# A rumen, animal and farm systems evaluation of fodder beet when used to supplement ryegrass during lactation

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A thesis by manuscript

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Anita Fleming

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Lincoln University

2020

## Thesis by manuscript

This Thesis by manuscript contains a set of manuscripts which have either been peer-reviewed and published or prepared for submision.

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February 2019

# Manuscripts prepared and submitted arising from this thesis and statement of the authors' contribution

**Inclusion of fodder beet in New Zealand grazing dairy systems; a paradigm for increased environmental, economic and animal welfare risk**

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*AF: conceptualisation, investigation, writing the original draft. GE: Conceptualisation, project administration. R.B: Conceptualisation, review and editing the draft. P.G: Conceptualisation, review and editing the draft.*

*Submitted for publication in the Journal of Applied Animal Science (July 2020).*

**Milk production and milk fatty acid composition of grazing dairy cows supplemented with fodder beet**

**(Appendix A.1)**

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*Manuscript published in the New Zealand Journal of Animal Science and Production, 2018 (78:6-10)*

**Supplementation of spring pasture with harvested fodder beet bulb alters rumen fermentation and increases the risk of sub-acute ruminal acidosis during early lactation**

**(Appendix A.2)**

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Published in Animals (June 2020)

**Rumen function and grazing behaviour of early-lactation dairy cows supplemented with fodder beet bulb**

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Accepted for publication in the Journal of Dairy Science (October 2020)

***In vitro* fermentation of fodder beet root increases cumulative gas production of methane and carbon dioxide.**

*(Appendix A.3)*

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Published in Livestock Science (August 2020).

**Modelling feeding strategies to improve milk production, rumen function and discomfort of the early lactation dairy cow supplemented with fodder beet.**

**(Appendix A.4)**

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Published in The Journal of Agricultural Science (August 2020).

**Fodder beet to support early and late lactation milk production from pasture, is it worth the risk?**

**(Appendix A.5)**

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AF: Conceptualisation, model parametrisation and analysis, data interpretation and writing. RB: Conceptualisation, supervision and editing. DD: Conceptualisation, supervision and editing. GE Conceptualisation and supervision. PG: Conceptualisation, supervision and editing.

Published in Agricultural Systems (November 2020)

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

# A rumen, animal and farm systems evaluation of fodder beet when used to supplement ryegrass pastures during lactation

by

Anita Fleming

The purpose of this thesis was to identify the functional changes at the rumen, individual animal and whole-farm scale when FB is used to supplement a ryegrass-based sward during lactation. A review of the literature (Chapter 2) explored the potential of FB to improve the feed base of the farm system and advance low infrastructure grazing systems which are common to New Zealand. However, the review also suggested greater biological, tactical and financial risk may be associated with growing and feeding FB on the milking platform, and the potential net advantage/disadvantage and had not been well defined in grazing dairy systems. Of primary concern was the incidence of acute and sub-acute ruminal acidosis (SARA) which has been underestimated across the herd in confinement and pastoral dairy systems worldwide. The incidence of SARA in cows transitioned to FB using industry-approved methods, and alternative FB feeding strategies to reduce SARA was highlighted as an area requiring further evaluation.

In the first experiment (Chapter 3), the effect of feeding FB during early lactation on milk production and milk fatty acid composition of grazing dairy cows was explored. Sixty Friesian × Jersey cows were blocked into six groups of 10 cows, and groups randomly allocated to three replicates fed either 18 kg DM/day of ryegrass herbage (H), or 14.4 kg DM/day of ryegrass herbage + 4 kg DM of harvested FB bulbs (FBB). There was no advantage to milk production when 30% of a ryegrass-based herbage diet was substituted for FB bulb although, this also indicated that FB might provide an adequate substitute for herbage during periods of feed deficit. Supplementation of herbage with FB increased ($P < 0.001$) *de novo* synthesis of saturated fatty acids (particularly; lauric, myristic and palmitic acids) and reduced substrate availability of unsaturated fatty acids for ruminal biohydrogenation which reduced ($P < 0.001$)

the content of long-chain and unsaturated products in milk. While the sward's chemical composition differed between treatments, the fatty acid content of herbage was not different ($P > 0.1$). The altered biohydrogenation end-products in milk and the high soluble-carbohydrate content of FB compared with herbage indicated altered rumen microbial communities and rumen function.

The second experiment (Chapters 4 & 5) was split into two chapters to evaluate two objectives. The first objective was to assess the industry recommended method for transitioning lactating dairy cows (+ 0.5 kg DM FB/day) to moderate (40% DMI) amounts of FB on changes in rumen fermentation, pH and risk of SARA. In a cross-over design, eight rumen cannulated cows during early lactation were fed one of two diets either; herbage only (HO) or 60:40 ryegrass herbage + FB bulb (FBH). Response variables were analysed as a 3x2 factorial arrangement of FB adaptation stage (Stage 1: transition day 1-12, Stage 2: adaptation day 13-17, Stage 3: post-adaptation day 18-20) and dietary treatment. Two animals experienced severe SARA (pH < 5.6 for >180 min/d), one during each period, they were closely monitored but were able to self-correct rumen pH without intervention. Across each treatment, the FBH diet increased estimated DMI (measured by calibrating sward height with sward mass), but milk production was similar to the HO diet. Ruminal pH of cows fed FBH declined below HO between 0100 h and 1200 h each day even during stage 3 of adaptation, which may have reduced the microbial degradation of structural carbohydrates and limited the milk response to FB. The large content of water-soluble carbohydrate content of FB prevented rumen pH from stabilising within 20 days of adaptation and elevated the risk of SARA in specific individuals. An extended period of transitioning and low FB allocation may be needed to prevent the risk of SARA grazing dairy cows supplemented with FB.

The objective of Chapter 5 was to evaluate the effect of supplementing spring ryegrass with moderate amounts (40% of total DMI) of FB on digestive and ingestive processing. We hypothesised that the decline of ruminal pH caused by supplementing ryegrass with FB would reduce the rumen function and microbial degradation of ryegrass. Following day 20 of adaptation (Chapter 4), the eight ruminal cannulated cows' rumen contents were removed at 0000 h, weighed and returned to the rumen and cows were fasted for ~10 h overnight before rumen contents were again removed and weighed. Samples determined particle comminution, pools of fermentation-end products and fractional neutral detergent fibre degradation between each bailing session. Minced samples of ryegrass and fodder beet were

incubated separately, *in sacco* over 20 h on day 20 of each period (between 0400 – 0000 h), to evaluate DM disappearance. Each cow's total jaw movement was recorded on day 16 and 18 of adaptation to FB to identify changes in behaviour (grazing, ruminating and idling) and oral processing (mastication and prehension). While calibration of pasture mass form height reported in Chapter 4 indicated the FBH treatment consumed greater DMI than HO, estimation of DMI from energy output in maintenance, milk production and loss of body condition indicate DMI between treatments was similar ($P > 0.10$). In addition, the rumen pool of DM, ADF and NDF measured at the first (0000 h) rumen bailing, also reflected DMI when calculated from animal output and maintenance. Cows fed FBH spent 86 min/day longer ruminating and chewing intensity while ruminating increased 38% compared with those fed HO, while grazing time declined 20 min/kg DM of FB eaten. While the fractional degradation of neutral detergent fibre was similar between treatments, the FBH diet reduced the total VFA pool compared with HO following fasting (3.67 versus 4.03 mol), due to reduced ruminal concentrations of acetate and propionate. Despite greater rumination and chewing intensity, the rumen pool of large particles (> 2 mm) following fasting, declined 28% in cows fed FBH compared with those fed HO. *In sacco* DM disappearance of ryegrass following 20 h of incubation also declined 19% ($P < 0.01$) in the FBH treatment. The decline of VFA pool, reduced particle comminution and DM disappearance of ryegrass *in sacco* support the hypothesis that supplementing grazing dairy cows with moderate (40% of DMI) amounts of FB reduces the microbial activity of the rumen and limits the milk response to FB. The results suggest minimal advantage and high risk to rumen function and animal welfare of individual cows supplemented with FB.

The third experiment (Chapter 6) evaluated the effect of a combined substrate containing ryegrass and increasing proportions of FB bulb (0, 15, 30 and 50 % of DM) on cumulative gas production and fermentation-end products *in vitro*. The objective of this study was to evaluate the dose-dependent response to supplementing ryegrass with FB bulb on the formation of fermentation end-products and gas production in 100 ml glass syringes. The total gas accumulated increased with the proportion of FB incubated ($P < 0.05$). The concentration of butyrate and propionate increased, while the concentration of acetate declined ($P < 0.01$), following 24-h of incubation. Production of carbon dioxide ($CO_2$) formed from buffering VFA and methane ($CH_4$) formed from fermentation, were calculated using stoichiometry. The percentage of $CH_4$ declined yet, the total accumulation of $CO_2$ and $CH_4$ increased with the

amount of FB included in the substrate. The effect of treatment on gas production diminished ($P > 0.10$) when the greater OM content of FB was accounted for, which indicate that while FB may reduce fractional $CH_4$ emissions, total methane emission may increase compared with ryegrass, due to the greater fermentable organic matter content of FB bulb.

Chapter 7 aimed to characterise changes of rumen pH, milk production and total discomfort from FB and define practical feeding strategies of a mixed herbage and FB diet. The deterministic, dynamic, and mechanistic model, MINDY, was used to compare a factorial arrangement of FB allowance, herbage allowance (HA), and allocation time. The FB allocations were 0, 2, 4 or 7 kg DM/cow per day (0, 2, 4 and 7FB, respectively) and HA were 18, 24 or 48 kg DM/cow per day above ground. All combinations were offered either in the morning or afternoon or split across two equal meals. Milk production from 2FB diets was similar to control but declined 4, and 16% when FB increased to 4 and 7 kg DM. MINDY predicted that 7FB would result in SARA and that rumen conditions were sub-optimal even at moderate FB allocations (pH < 5.6 for 160 and 90 min/d, 7 and 4FB respectively). Pareto Front analysis identified that splitting the 2FB diet into two equal meals fed each day alongside daily HA of 48 kg DM/cow provided the best compromise between high milk production and low total discomfort. However, due to low milk response and high risk of acidosis, we conclude that FB is a poor supplement for lactating dairy cows.

In Chapter 8 a multi-component, whole-farm modelling approach was used to predict milk solids (MS) production and the economic farm surplus (EFS: operating surplus – adjustments) over two seasons (2016-2018) for an irrigated farm in Canterbury (South Island) and a non-irrigated farm in the Waikato (North Island), of New Zealand. The financial risk of the dairy business was measured using stochastic modelling in which the ratio between mean return on assets (ROA) minus an assumed 5% risk-free ROA, and the standard deviation of ROA was calculated from 300 combinations of climate, milk and feed price, land appreciation and interest rate. Four scenarios of autumn and spring supplementation of pasture were considered at each geographical location; imported maize silage (Base), maize silage crop grown on the platform (MSC), FB crop is grown on the platform (FBC) and FBAC a FB crop with an outbreak of acute (1% stock fatality) and SARA (5% decline of feed intake). Crop yield of FB increased with irrigation (21 versus 23 t DM/ha; irrigated and dryland, respectively) and was greater than maize silage (19 versus 21 t DM/ha; irrigated and dryland respectively). The DM yield of maize silage increased with the dryland system due to the warmer climate in the

Waikato region of New Zealand (NZ). The MSC scenario improved EFS 5.8 % compared with Base when introduced to either the irrigated or the dryland system. The predicted response to MSC reflected a combination of greater milk production, lower feed expenses and shorter crop rotation compared with either Base, FBC or FBAC. While FBC increased EFS by 4.8% compared with Base under irrigation, EFS was similar to Base under dryland conditions ($2,711 and $2,759/ha, respectively). The limited advantage of growing FB under dryland conditions reflect reduced herbage supply due to the extended crop duration of FB compared with maize silage (14 versus 11 months between grazing of herbage). Model predictions suggest FBAC would reduce EFS by 6.5% (irrigated) and 7.1% (dryland) compared with Base, due to reduced milk production and livestock sales. In the absence of any adverse health risks, farm performance from supplementing FB crop was comparable to maize silage under irrigated conditions. However, in dryland conditions, and when the potential economic cost of acute and sub-acute ruminal acidosis is considered, there is little advantage from growing FB on the milking platform.

While there is some support that minor allocation of FB with herbage will improve animal production, the novel methods of feeding and grazing FB in NZ increase animal welfare risk of individual animals within the herd, preventing the elimination of SARA risk when feeding FB to support lactation. Besides a few recent studies, previous research of FB feeding systems in NZ has focused on the herd as an experimental unit. However, the dynamics of feeding FB to individuals within the herd are variable, and the risk of SARA caused by supplementing ryegrass-pastures within commercial dairy systems of NZ may be underestimated. Further research should focus on factors responsible for individual risk to SARA such as competition, grazing and feeding behaviour, epithelial function, and morphology and rumen fermentation. Attention is needed when feeding FB to large herds in minimal infrastructure systems which prevent individualised feeding of FB as the variation of FB and herbage intake between individuals and days alter the allocation of FB to the remaining individuals within the herd. The results from this thesis suggest feeding small amounts of FB may help improve milk production and reduce feed deficits; however, the risk of SARA increases with FB allocation. Profit comparisons indicate limited financial incentive to growing FB on the milking platform to supplement ryegrass during early and late-lactation compared with lower-risk alternatives such as maize silage. In conclusion, from a rumen, individual

animal and farm systems perspective, there is no advantage to supplementing grazing dairy cows with fodder beet to support lactation.

**Keywords:** fodder beet, milk production, grazing dairy cows, milk fatty acids, rumen fermentation, rumen pH, fractional rumen degradation, particle comminution, cumulative gas production, enteric methane emission, feeding strategies, minimum total discomfort, whole-farm modelling, farm systems, risk.

# Acknowledgements

I would first like to acknowledge Lincoln University for funding my research and the substantial support and advice provided by each of my supervisors during the past four years it has taken me to complete this collection of manuscripts. I would particularly like to thank Grant Edwards, who first introduced me to this research topic and the possibility of completing a thesis. While Grant was not able to finish my thesis as a lead supervisor, I was fortunate to be taken under the wing of Pablo Gregorini, who very quickly challenged my ideas and has opened a world of opportunity in ruminant nutrition and mechanistic modelling. I am sincerely grateful for his constant support, guidance and faith in my abilities. I have also been fortunate to be supervised by Racheal Bryant, who has quietly transformed my writing skills and is always patient and quick to provide detailed and grounding feedback, particularly, when I get carried away. Dawn Dalley's input has also been crucial to developing these papers; her industry perspective and feedback are always appreciated. I could not have completed this thesis without the help of farmworkers at both LURDF and ADRDS, their contribution, particularly during the first experiment was invaluable. I am equally indebted to the Lincoln university analytical team who have helped over the years with laboratory analyses - Shuang, Jenny, Barry and Rosy.

I have been fortunate to meet many other PhD students throughout this journey, but in particular, thanks to – Konagh, Kelly, Matt, Christopher, and Cameron. The experiment conducted in Chapter 4 was very intensive – from rumen sampling in the horizontal rain to rumen bailing at all hours - and I am still very grateful for their dedication. To all I have gotten to know while completing this project, I wish you all the best for the future!

I am privileged to be a part of a very special village of family and friends which have been supportive throughout this process; my family - Sue, Vaughan, Alex and Vicky, and in-laws - Rosalie and Graham who have been gracious enough to let me 'experiment' some of my ideas in our own dairy business. Finally, I would not have completed this project without the constant encouragement and support of my better half – James who has been there without fail throughout this long-winded journey, I look forward to moving on to the next chapter of my career and life with you and the adventures which will undoubtedly follow.

# Table of Contents

# List of Tables

# List of Figures

# Chapter 1

# General Introduction

The New Zealand pastoral dairy industry relies heavily on perennial ryegrass (*Lolium perenne*, PRG) and white clover (*Trifolium repens*) pastures for meeting the energy demands of lactation. Since 2000, the amount of milk processed in NZ has increased by 37%. While the number of herds has declined 17%, the average herd size has increased from 251 cows to 431 cows, and milk solids (fat + protein) production per cow have also increased 16% within the last 20 years (DairyNZ, 2018b). Genetic selection for milk production and irrigation have intensified NZ pastoral dairy systems. However, this has increased debt levels and reduced fertility (Lucy et al., 2009; Roche et al., 2009). It has become apparent that all modern dairy cows experience some form of negative energy balance (when energy output for maintenance and production exceed energy intake: NEB), that increases the mobilisation of adipose tissue and delays the return of the oestrous cycle, postpartum (Roche, 2006). While the target interval between calving is 365 days in NZ, the average calving interval has increased across all breeds from 368 days in 2000 to 371 days in 2018 (DairyNZ, 2018b). Genetic selection for milk production is an underlying factor for the increased postpartum NEB and reduced fertility of dairy cows, which has been experienced globally (Butler and Moore, 2018). However, pastoral dairy systems face an additional challenge as a 365 d calving interval and tight 6-week calving period is needed to balance the seasonal pasture supply with nutrient requirements for lactation.

The seasonal supply of herbage is characterised by a deficit of pasture growth from late autumn to early spring and often during summer months when low rainfall and high evapotranspiration reduce pasture growth. Depending on the system's grazing intensity, herbage deficits are balanced by surplus growth between spring to early summer and autumn. Therefore, high energy readily digestible supplement feeds can help complement the seasonality of herbage growth. The nutrient content of pasture is also seasonal and often provides excess fibre, crude protein, and insufficient soluble carbohydrates needed for modern dairy cows to achieve their genetic potential for milk production (Burke et al., 2002;

Kolver, 2003). However, pastoral dairy systems maintain profit margins by minimising variable costs such as feed as the NZ agricultural industry is unregulated, and the price of milk also fluctuates. Feeds such as; pasture, lucerne (*Medicago sativa L.*), and maize (*Zea mays L.*) silage, palm kernel extract (a by-product of the palm oil industry), and cereal grains, can be purchased to mitigate spring and autumn herbage deficits.

The intensity of New Zealand dairy systems is defined by the amount of feed imported onto the milking platform (the area used to feed dairy cows throughout lactation). System intensities range from system 5, where > 40% of feed is imported (including support blocks and feed grazed off-farm during the winter dry-period) to system 1 of 0% feed imported (cows wintered on-farm). The number of system 1 farms has declined by 30% between 2000 and 2015 (currently < 10% of NZ dairy farms), while the number of system 5 farms has increased only 1% and as of 2015, represent 4-9 % of NZ dairy farms. Approximately 40-45% of NZ dairy farms are system 3 (10-20% of feed is imported), and 20-25% are system four ( 30-40% of feed is imported) (Fausett et al., 2015). While minimal infrastructure is typical for level 3-4 systems, capital investment in irrigation, effluent storage, and effluent disposal have increased land value, and the debt to asset ratio has also increased 9%, between 2008 and 2017 (DairyNZ, 2018a). The increasing debt to asset ratio enhances the need for dairy systems to remain profitable and service debt, despite the unstable operating environment that NZ dairy producers are exposed to (climate, supplement/feed price, milk price).

New Zealand dairy farmers face a trade-off between meeting environmental and societal expectations and servicing substantial debt levels. In this respect, fodder beet (*Beta vulgaris L*, FB) has become a popular feed source in the NZ dairy industry as it is high yielding (Chakwizira et al., 2014; Chakwizira et al., 2016) and highly utilisable when grazed *in situ*. The bulb of FB also contains low amounts of nitrogen that complement the high protein content of ryegrass and can be a low-cost feed < 10 c /kg DM (Gibbs, 2014; Dalley et al., 2019). However, the reduction of winter grazing area and increased DM yield will increase stocking density, damaging soil structure and cause sediment run-off. The high water-soluble carbohydrate content (WSC) and low crude protein (CP) content of FB bulb represent an additional risk to animal welfare and health (Waghorn et al., 2018; Pacheco et al., 2020). While correct transitioning and careful feeding management of FB are reported to prevent the occurrence of acute and sub-acute ruminal acidosis from FB (Gibbs, 2014), the

development of SARA following transitioning has still been reported in late-lactation dairy cows (Waghorn et al., 2019).

Furthermore, the evidence available suggests the milk response to FB supplementation is limited despite the large amounts of readily fermentable WSC in FB bulb (Waghorn et al., 2019). The decline of ruminal pH due to feeding dairy cows FB may also reduce the ruminal biohydrogenation of poly-unsaturated fatty acids, increasing their milk content. However, the implications of supplementing lactating dairy cows with FB on the qualitative components of milk have also not been analysed. Therefore, the evaluation of the advantages and limitations to rumen, animal and farm systems function in NZ pastoral systems is required to enhance management practices and animal welfare when FB is used to support early or late-lactation milk production.

## 1.1 Objectives

The main objective of this thesis was to investigate inter-scalar impacts of supplementing a ryegrass-dominant sward with FB by looking at the changes within the rumen, the individual animal and the whole farm system.

More specifically, the objectives were to:

**Objective #1:** Outline the potential advantages of feeding fodder beet to support early and late-lactation milk production in New Zealand pastoral dairy systems.

**Objective 2**: Determine the effect of supplementing FB to a ryegrass-based diet on milk production and milk fatty acid composition of grazing dairy cows at peak lactation.

**Objective 3:** Evaluate the time-dependant adaptions of rumen function, fermentation, dry matter intake, and risk of sub-acute ruminal acidosis in lactating dairy cows supplemented with FB, using industry-approved methods of FB transitioning.

**Objective 4:** Evaluate feeding managements that reduce the risk of ruminal acidosis and maximise the individual animal response (milk production, rumen function and discomfort) to FB supplementation.

**Objective 5:** Evaluate profit margin, business and environmental risk in response to growing and feeding fodder beet on the milking platform and evaluate cost ruminal acidosis from FB.

## 1.2 Hypothesis

The following hypotheses were tested using three experiments that evaluated the milk response, milk composition, rumen function, and transitioning efficacy when supplementing perennial ryegrass with fodder beet. Two modelling studies analysed feeding methods to assess the individual animal response, profit margin, and risk of the whole farm system when FB is grown and fed to dairy cows on the milking platform.

**Hypothesis #1:** *Supplementing ryegrass with fodder beet during early-lactation will increase water-soluble carbohydrate intake, early-lactation milk production and the poly-unsaturated fatty acid content of milk compared with cows fed herbage only.*

**Hypothesis #2:** *The marginal milk response to FB will decline, and risk of sub-acute ruminal acidosis will increase relative to the amount of FB used to supplement a perennial ryegrass pasture during lactation.*

**Hypothesis #3:** *Fodder beet will reduce rumen pH, increase fermentation kinetics and the formation of volatile fatty acids specifically, butyric and propionic acid compared with a control diet of herbage only.*

***Hypothesis #4*** *The use of industry-approved methods for transitioning lactating dairy cows to moderate (~40% daily intake) amounts of FB and ryegrass will prevent ruminal acidosis from FB.*

**Hypothesis #5:** *Supplementing ryegrass with moderate (<40 % DM intake) amounts of FB will reduce rumen pH and fermentation of ryegrass, grazing time and alter ingestive behaviour.*

**Hypothesis #6:** *A combination of feeding time, frequency and amount of either fodder beet and ryegrass herbage can be used to improve the marginal milk response and reduce the risk of ruminal acidosis in cows supplemented with fodder beet.*

**Hypothesis #7:** *The incorporation of Fodder beet on the milking platform will help mitigate seasonal herbage growth and support early and late-lactation milk production, but increase risk exposure and compared with alternative forage crops or imported supplement.*

Table 1.1 Thesis structure including objectives and hypothesis of the research completed within this thesis

| | | |
|---|---|---|
| Chapter 1 | General Introduction | |
| Chapter 2 | Literature Review | Hypothesis # 1-7 |
| Chapter 3 | **Objective:** Determine the effect of substituting the herbage of grazing dairy cows with FB on milk production and milk fatty acid composition | Hypothesis # 1 & 4 |
| Chapter 4 | **Objective:** Quantify the time-dependent changes to milk production, milk fatty acid composition, rumen function and fermentation when grazing, early-lactation dairy cows are supplemented with moderate amounts of FB. | Hypothesis # 1, 2, 3, & 4 |
| Chapter 5 | **Objective:** Evaluate the effect of supplementing spring ryegrass with a moderate amount (40% of total DMI) of harvested FB during early lactation on rumen function, oral processing, grazing behaviour and rumen digestion. | Hypothesis # 5 |
| Chapter 6 | **Objective:** Define how the proportion of DM supplementation of a ryegrass-based herbage diet with FB root alters the formation of fermentation end-products and gas production *in vitro*. | Hypothesis # 2 & 3 |
| Chapter 7 | **Objective:** Assess the role of feeding management for reducing the risk of ruminal acidosis, maximising the individual animal response (milk production, rumen function and discomfort) from FB when used to supplement a ryegrass-based herbage. | Hypothesis # 2 & 6 |
| Chapter 8 | **Objective:** Evaluate profit margin, biological and financial risk when growing and feeding fodder beet on the milking platform of both irrigated and dryland pastoral-dairy systems. | Hypothesis # 7 |
| Chapter 9 | General discussion | Hypothesis # 1-7 |

# Chapter 2

# Inclusion of fodder beet in New Zealand grazing dairy systems; a paradigm for increased animal welfare, economic and environmental risk

*Submitted for publication in the Journal of Applied Animal Science (July 2020)*

## 2.1 Abstract

Despite extensive use of fodder beet (FB) in New Zealand to compliment seasonal herbage growth, research of physiological, environmental and financial implications of growing and feeding FB to support lactation is limited. Increasing concern for animal welfare of cows fed FB has warranted a holistic review of the potential impacts on animal welfare, farm profit and environmental outcomes when FB is used in pastoral dairy systems. While large yields (>20 t DM/ha) and high rate of utilisation (90%) of FB are attractive to farmers; expensive establishment costs, high substitution rates and low milk responses to FB may restrict profit margin. Limited milk production may reflect sub-optimal rumen pH and sub-acute ruminal acidosis, which reduces; ruminal fermentation, animal intake, milk production, and can cause acute and chronic impairment of rumen endothelial function. Winter FB feeding programmes may further impair animal welfare by increasing mineral imbalance and the incidence of metabolic disorders such as hypophosphatemia, hypocalcaemia, hypomagnesemia, ketosis and hepatic lipidosis. There are several factors which may reduce animal welfare of lactating and non-lactating dairy cows that are fed FB. While recent research has focused on grazing FB during late lactation, further definition of FB feeding management pre and postpartum is needed, when the risk of nutritional and metabolic disorders are greatest. Fodder beet is a low crude protein feed yet, large crop yields increase stocking density and nutrient leaching, while also causing extensive soil erosion during autumn and winter periods. Given the limited information that has formally analysed the potential advantage or risk when FB is used to support pastoral dairy systems, we advise dairy producers to proceed with caution.

## 2.2 Introduction

New Zealand pastoral dairy systems require additional forages and imported supplemental feed to counteract seasonal herbage growth. While the temperate climate in NZ is optimal for

pastoral farming from spring to mid-autumn (with the addition of irrigation in some regions), imported supplements and forage crops are needed to support milk production and liveweight gain between late autumn and early spring. However, the arable area available for growing energy-dense crops such as barley (*Hordeum vulgare, L.*) or maize (*Zea mays, L*) grain to supplement pasture is limited, and the cost of imported supplements is expensive and variable in NZ (Ramsbottom et al., 2015). Market conditions are further convoluted by the NZ agricultural industry's deregulated nature, which also causes milk prices to fluctuate (Dillon et al., 2005). The unstable and often low, differential between milk and feed price constrain profit margins (net income – farm working expenses) (McCall and Clark, 1999; Dillon et al., 2005). Thus, low input pastoral dairy systems which use annual forage crops to mitigate seasonal herbage supply, are popular in NZ.

In recent years, New Zealand dairy producers have come to rely on fodder beet (FB; *Beta vulgaris L.*) to increase body condition score (BCS), prepartum. Compared with an alternative winter forage such as kale (*Brassica oleracea*), FB has the potential to grow large yields (>20 t DM/ha) of a highly utilisable bulb (97.6%) (Saldias and Gibbs, 2016) in addition to ~4 t DM/ha of a leaf, which senesces over winter. The versatility to graze or harvest FB is a novelty which has quickly led to the widespread use of FB to counteract the seasonality of herbage growth during late-lactation and winter, while residual winter crop can be defoliated, harvested and fed during early-lactation. However, there are several risks associated with growing and feeding FB. The low proportions of fibre, protein and minerals (calcium and, phosphorous) and high concentration of water-soluble carbohydrates (Dalley et al., 2017), are risk factors for metabolic disorders such as ruminal acidosis. Ruminal acidosis can have several immediate and long-term repercussions to animal production and welfare although, the extent of their impacts in pastoral dairy systems have not been well defined (Owens et al., 1998; Bramley et al., 2008; Zebeli and Metzler-Zebeli, 2012a). Recommendations for achieving prepartum BCS targets from FB (~5.0, scale: 1-10, where 1 is emaciated and 10 is obese), involve transitioning non-lactating dairy cows up to appetite levels of FB (10-12 kg DM/day) over a short 14-d period and feeding minor portions (<3 kg DM/cow/day) of straw or silage (Gibbs, 2014). While current industry recommendations suggest that straw or silage should account for $\geq$ 30% of DMI (DairyNZ, 2017), transitioning cows to appetite amounts of FB may reduce intake rate, VFA accumulation and the risk of ruminal acidosis, should temporary

electric fencing fail (Gibbs, 2011). However, the efficacy of appetite or restricted feeding methods of FB for preventing nutritional disorders from FB has not been reviewed.

NZ dairy producers have readily adopted fodder beet because of the potential yield advantages and the reduced area needed to graze non-lactating dairy cows during winter. For example, 5.4 ha of kale will support 100 cows over the eight-week, non-lactating, winter period (assuming: 14 t DM/ha, 60 % utilization, and 70:30 kale and straw), compared with the 2.2 ha that will support the same animals using FB (assuming: 25 t DM/ha, 80% utilization, and 70:30 FB and straw). However, the reduction of winter crop area due to growing FB will also increase stocking rate (from ~ 10 to 25 cows/ha, based on the above assumptions) which may increase soil erosion from pugging and cause soil compaction below the cultivatable level (Drewry and Paton, 2005). Furthermore, a comprehensive review of the system implications of growing fodder beet on the milking platform (the land area which supports lactation) with particular reference to milk production, composition, animal welfare, health, and the environment, is warranted.

This review aims to describe the effects of feeding fodder beet to dairy cows on milk production and composition, nutritional imbalances that affect animal welfare while also discussing financial, risk and environmental outcomes.

## 2.3 Milk Production

### 2.3.1 Milk response to fodder beet

Milk production income represents over 90% of the total farm revenue in NZ dairy businesses (DairyNZ, 2018a). Therefore, importing or growing expensive supplemental feeds must increase milk production and profit above the base diet to justify the additional risk. The milk price in NZ is based on milk solids (MS: fat + protein); therefore, changes in milk composition and yield of milk components can directly impact farm revenue. However, the milk response of whole milk or MS to FB is variable and often low, ranging between -0.6 to +0.6 kg MS/kg DM of FB (Table 2). Roberts (1987) reported that supplementing autumn calving cows with either 0, 2, or 4 kg DM of FB during winter did not affect daily milk yield compared with herbage only counterparts. In agreement, the milk response of early lactation dairy cows declined from +0.23 to +0.08 kg milk/kg DM of supplement when cereal grains were replaced with pelleted fodder beet (Ferris et al., 2003). Despite greater DMI from cows fed FB, milk yield was unaffected, suggesting reduced feed conversion efficiency (Ferris et al., 2003).

Substitution of pasture for FB increased milk production compared with a pasture only diet (25.9 versus 23.0 kg/day). Still, it decreased milk production compared with cows supplemented with maize silage (26.4 kg/day), or brewer's grain (26.7 kg/day) (Phipps et al., 1995). Dalley et al. (2019) recently reported that FB increased milk yield compared with maize silage when fed as < 25 % DMI. On the other hand, moderate (< 30% DMI) supplementation of ryegrass with FB during late-lactation (Alabi, 2019) was not advantageous to milk production compared with a control supplement of ryegrass silage.

While the milk production response to FB supplementation appears to be limited, milk composition changes have revealed some unique results. The proportion of milk fat and protein increased relative to the level of FB fed during early-lactation (Table 2) and compared with the control silage-only diet, the yield of MS increased 6.4 % and 7.0 % when silage was supplemented with 2 and 4 kg DM of FB, respectively (Roberts, 1987). The yield of milk fat increased by 2.9 g/day when FB was fed during mid-late lactation (Fisher et al., 1994). Furthermore, the yield of milk fat and protein has been reported to increase by 16 and 10 g/d, respectively for up to five weeks postpartum when non-lactating cows were fed FB prepartum (Keogh et al., 2009). Similar responses have been identified in cows fed a total mixed ration (TMR) supplemented with either brewer's barley, FB, potatoes (Eriksson et al., 2004), or FB compared with grass silage, maize silage, or brewers grains (Phipps et al., 1995). However, in the research conducted using NZ grazing and feeding conditions, the MS response to supplementing a ryegrass pasture with FB is low compared with either maize silage supplements or a herbage only diet (Alabi, 2019; Dalley et al., 2019; Waghorn et al., 2019). While there may be some opportunity to improve MS production with FB in ration-based systems, further research in the context of NZ pastoral dairy systems is needed to define the MS response to FB when it is used to supplement herbage.

One explanation for the low milk response could relate to variation in the gross energy content of FB. The ME content of FB bulb is greater than maize silage and is commonly considered to be similar to cereal grains ~12 MJ ME, assuming standard feeding assumptions for crops and forages (Gibbs, 2014). However, Clark et al. (1987) identified that the GE content of the bulb was relatively low (16.0 MJ GE/kg DM) compared with forages (18.8 MJ GE/kg DM, (AFRC, 1993). A recent analysis by Waghorn et al. (2018) present similar conclusions, as the GE of FB bulb (16.4) is less than straw (18.0) and pasture silage (18.9 MJ GE/kg DM). Based on the apparent DM digestibility reported by Waghorn et al. (2018), ME intake declined with

increased FB allocation (193, 182, and 168 MJ ME/day 0 %, 23 %, 45 % of daily intake as FB, respectively). Therefore, the GE content of FB may explain the lower than expected milk response. However, increasing FB allocation from 23 to 45 % of DMI also reduced the digestibility of NDF by 10.3 % which may further indicate the limited milk response to FB may be due to changes in rumen fermentation.

Altered proportions of protein or fat within milk are a consequence of dietary composition and animal metabolic state. Therefore, the high WSC content of FB (Table 2.1), could increase the production of glucogenic precursors such as propionic acid, which can encourage microbial protein synthesis. Eriksson et al. (2004), reported that the inclusion of FB limited CP and ME intake; however, this did not affect amino acid supply to the duodenum, indicating that FB increased microbial protein synthesis. Ferris et al. (2003) reported the concentration of urine purine derivatives increased, which similarly suggest the low crude protein content of FB bulb is offset by an increase in microbial protein supply (Roberts, 1987; Fisher et al., 1994; Ferris et al., 2003). However, the estimation of microbial N growth in late lactation dairy cows does not support this conclusion as microbial N declined numerically in response to increased allocation of FB (15.53, 13.65, 12.17 g N/kg OM digested, 0, 23 and 45 % FB

Table 2.1. Effect of the dietary proportion of fodder beet (FB) and days in milk (DIM) on milk yield, (kg/cow/day), milk solids yield (kg MS/ cow/day), proportion of milk constituents (fat, protein and lactose), milk response and substitution rate.

| Reference | Diet | HA[1] | FB % | DIM (days) | Milk (kg/day) | MS yield (kg/day) | Milk response[2] | Fat % | Protein % | Lactose % | Substitution Rate[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Roberts, 1987) | Silage + FB | 8.7 | 12.2 | >150 | 23.3 | 1.828 | +0.15 | 4.42 | 3.45 | 4.79 | 0.65 |
| (Roberts, 1987) | Silage + FB | 7.2 | 23.1 | >150 | 23.9 | 1.94 | +0.23 | 4.59 | 3.53 | 4.73 | 0.70 |
| (Ferris et al., 2003) | Silage + FB+ 30% concentrate | 8.6 | 26.1 | 50 | 20.8 | 1.48 | +0.44 | 4.04 | 3.06 | 4.95 | - |
| (Ferris et al., 2003) | Silage +FB + 53% concentrate | 7.2 | 18.8 | 50 | 26.8 | 1.97 | +0.29 | 4.03 | 3.39 | 4.98 | - |
| (Ferris et al., 2003) | Silage + FB + 70% concentrate | 5.4 | 13 | 50 | 28.6 | 2.15 | +0.008 | 4.06 | 3.54 | 5.02 | - |
| (Phipps et al., 1995) | Silage + FB | 10 | 21.7 | 21-140 | 25.9 | 1.96 | +0.76 | 4.32 | 3.24 | 4.69 | 0.58 |
| (Fisher et al., 1994) | Silage + FB low protein | 6.3 | 21.3 | 44 | 22.5 | 1.60 | -0.41 | 3.88 | 3.11 | 4.85 | 0.59 |
| (Fisher et al., 1994) | Silage + FB, med protein | 6.8 | 21.5 | 44 | 23.8 | 1.73 | +0.08 | 4.03 | 3.21 | 4.83 | 0.45 |
| (Fisher et al., 1994) | Silage + FB, high protein | 6.7 | 22.4 | 44 | 26.5 | 1.88 | +0.08 | 3.96 | 3.22 | 4.94 | 0.56 |
| (Eriksson et al., 2004) | Lucerne silage + FB and potato mix (80:20) | 12.7 | 19.5 | 115 | 21.8 | 1.67 | -0.43 | 4.58 | 3.15 | 4.77 | |
| (Dalley et al., 2019) | Pasture + FB | 12.8 | 23.8 | 224 | 11.3 | 1.10 | -0.05 | 5.54 | 4.31 | 4.71 | |
| (Dalley et al., 2019) | Pasture + FB | 10.6 | 35.0 | 224 | 10.7 | 0.98 | -0.14 | 5.07 | 4.34 | 4.66 | |
| (Waghorn et al., 2019) | Pasture + FB | 11.8 | 23 | 205 | 11.6 | 1.07 | +0.6 | 5.47 | 3.80 | 4.67 | - |
| (Waghorn et al., 2019) | Pasture + FB | 6.89 | 45 | 205 | 10.9 | 0.91 | +0.22 | 5.36 | 3.69 | 4.64 | - |
| (Alabi, 2019) | Pasture +3 kg FB | 18.0 | 20.4 | 66 | 20.9 | 1.89 | -0.63 | 4.8 | 3.8 | 5.0 | 1.5 |

[1] Herbage allocation, [2] kg milk/kg DM FB compared with control counterparts. [3] Decline of pasture intake kg DM/kg DM of FB

as DMI) (Waghorn et al., 2019). Furthermore, changes to milk protein contrast with reports of increased milk fat content, which is driven by the synthesis of lipogenic precursors; acetic and butyric acid, within the rumen. There is little evidence that FB improves microbial protein synthesis when used to supplement a ryegrass pasture during late lactation (Pacheco et al., 2020), which may reflect limited synchronization of readily fermented sugar and protein under grazing conditions (Hall and Huntington, 2008). The consumption of readily fermentable diets can reduce the ruminal formation of lipogenic precursors, reducing milk fat production (Stockdale et al., 1987; Dalley et al., 2001a). Fodder beet contains large proportions of readily fermentable sucrose (40% DM) which appear to favour the formation of butyrate in the rumen (Quin et al., 1980; Winzer et al., 1996; Oba, 2011). Acetate (~60 %) and propionate (~20-30 %) are the primary end-products of fermentation, while butyrate is primarily metabolised (~80 %) by the rumen epithelium (Allen, 1997). Therefore, the varying response across the available literature that describes the changes of fat and protein content of milk produced from cows fed FB could either reflect the physiological state of experimental animals or variation of the nutritional composition of the basal diet. Subsequently, further evaluation of the changes to milk composition and ruminal fermentation of cows that are fed FB at different stages of lactation is warranted.

### 2.3.2 Milk fatty acid profiles

Interest in milk fatty acid profiles arises from the awareness that poly-unsaturated fatty acid (PUFA) content in milk is associated with a range of product quality, and human health-related benefits (Bauman and Griinari, 2001; Bobe et al., 2003). Pasture contains significant quantities of omega-3 and omega-6 fatty acids, in the form of $\alpha$-linolenic acid (C18:3) and conjugated linoleic acid (CLA) isomers (predominantly C18:2 *cis*-9, *trans*-11 but also C18:2 *trans*-10 *cis*-12), respectively. However, microbial biohydrogenation of PUFA in the rumen reduces their abundance in milk. Collomb et al. (2004) examined milk FA profiles from cows fed either 1.4 kg of supplement (ground rapeseed, linseed or sunflower seed) or a control of *ad-libitum* hay and 15 kg of fresh FB. While not a direct analysis of the effect FB has on FA profiles, saturated fatty acids (SFA) were more concentrated compared with milk produced from alternative supplements. Saturated fatty acids have been associated with increased bio-hydrogenation, leading to increased methane production, which also implies adjustment of microbial activity within the rumen (Dewhurst et al., 2006). Therefore, information describing the effect of

supplementing pasture with fodder beet on milk fatty acid profiles may enhance knowledge of the effect on aspects of milk processing and milk quality.

The available research indicates insufficient information to conclude how supplementing pasture with FB will affect milk production and milk composition. The variable and often limited marginal milk response to FB may be a consequence of both physiological state and nutritional imbalance. Thus the evaluation of the changes to rumen function in lactating dairy cows is required.

## 2.4 Animal Welfare

### 2.4.1 Acute and sub-acute ruminal acidosis

The rumen exists in a symbiotic state, as resident microorganisms (bacteria, fungi, protozoa, and archaea) metabolise protein and carbohydrates producing specific short-chain fatty acids, microbial protein, and vitamins, which are needed for the ruminant's metabolism. A sudden dietary increase of readily fermented WSC and reduced supply of NDF can cause VFA to accumulate rapidly, reducing pH and disrupting the symbiotic relationship (Kolver and De Veth, 2002; Zebeli et al., 2012b), causing malaise (Kleen et al., 2003), reduced motility of rumen epithelial and secretion of saliva containing phosphate ($PO_4^-$) and bicarbonate ($HCO^{3-}$) buffers which neutralise pH (Owens et al., 1998). Ruminal acidosis can severely impair animal welfare as rumen acidity can damage the ruminal and intestinal wall, increase the incidence of laminitis, cause liver abscesses, decrease blood pH, and cause severe, fatal dehydration (Owens et al., 1998; Kleen et al., 2003). Ruminal acidosis is most commonly experienced during early-lactation in dairy cows fed starch-rich diets. However, recent (< 10 years) inclusion of FB in NZ dairy systems has increased the incidence of this nutritional disorder when FB is fed (during early lactation, late lactation, and winter) due to the large fraction of WSC and the low fibre content of FB bulb (Dalley et al., 2017). While total rumen VFA concentration of non-lactating cows fed FB to appetite was lower than those fed herbage only (Prendergast and Gibbs, 2015), Waghorn et al. (2018) reported 5 out of 8 non-lactating dairy cows developed clinical acidosis post-transitioning to a FB winter diet. However, further research describing the changes to the rumen function of dairy cows grazing different FB levels during winter is needed to further define the risk that FB poses to RA and animal welfare and develop feeding strategies that optimise animal production from FB.

Acute forms of acidosis can be fatal and represent a large potential loss of revenue from the farm business. However, sub-acute forms of acidosis also reduce farm revenue and reduce animal welfare (Plaizier et al., 2008). During SARA, rumen pH is reduced episodically throughout the day due to the combined effect of VFA accumulation and reduced buffering capacity (Zebeli et al., 2012a). Unlike acute RA, lactic acid concentrations of animals affected by SARA will be < 10mmol/L (Hibbard et al., 1995) and rumen pH is restored without intervention (Plaizier et al., 2008). The digestion of fibre and cellulose is inhibited at low pH due to reduced cellulolytic and fibrolytic bacteria (Owens et al., 1998; Kolver and De Veth, 2002). De Veth and Kolver (1999) reported the degradation of total DM, NDF, and non-structural carbohydrates declined 14, 11, and 5 %, respectively, when pH fell from 6.4 to 5.4 in-vitro. Growth of cellulolytic bacteria such as *B. Fibrisolvens*, *B. Sunccinogens*, *Ruminococcus albus,* and *Ruminococcus flavefaciens* are acutely sensitive to pH changes and were washed out of culture when pH declined just 0.25 of a unit (Russell and Dombrowski, 1980). The potential reduction of cellulolytic and fibrolytic activity at low rumen pH due to FB consumption is a major concern for grazing dairy cows, in which fibre (NDF and ADF) is a primary dietary component.

Ruminants that are exposed to periods of low rumen pH are also at risk of developing para-hyperkeratosis of the epithelium (Zebeli et al., 2012b), which limit the absorption of VFA, reducing production and increasing the susceptibility to RA, by reducing the ability of the rumen to absorb and neutralise VFA (Bull et al., 1965). Disorders of rumen mucosa and low rumen pH may also increase the permeability of larger metabolites such as lipopolysaccharide also known as endotoxin, a cell wall component of gram-negative bacteria (Zebeli et al., 2012b). Endotoxins are vaso-active and can cause inflammation of the corium leading to laminitis and lameness (Nocek, 1997). The accumulation of endotoxin in plasma can also stimulate hepatocytes to produce acute-phase proteins such as; lipopolysaccharide-binding protein, serum amyloid A and haptoglobin, which trigger a generalised systemic immune response (Gruys et al., 2005). Increased lameness and reduced immune status may have severe consequences to animal welfare and production in NZ, due to the extended distances cows are often required to walk and the large amounts of energy and protein expended to maintain immune function. However, the potential for FB to alter rumen epithelial function, and the incidence of laminitis in New Zealand dairy cows is unknown.

While the low fibre and high sugar content of a herbage and FB diet have the potential to cause SARA, the degree of insult (and implication to animal health) and level of prevalence across the NZ dairy herd is not easily quantified. Limited diagnostic tests, and minimal clinical symptoms, have made the task of correctly identifying SARA affected cows within a herd, a subject of discussion. Ruminal pH levels of < 6.0 (Plaizier et al., 2008), 5.5 (Garrett et al., 1999), 5.2 - 5.6 (Khafipour et al., 2009) or < 5.8 for more than 3 hours/day are all indicative of SARA (Zebeli et al., 2012b), and there is no singular agreed-upon threshold. These discrepancies may be related to diet variations, lactation stage/physiological state, sampling method, and sampling site in the rumen (Duffield et al., 2004; Plaizier et al., 2008). The duration that rumen pH is below a certain level, i.e., < 6.0 or 5.8 through the day is the most commonly used, although, this requires continuous monitoring which can be expensive for commercial use (Westwood and Lean, 2001).

Acute and SARA are a common risk in TMR systems in which readily fermentable carbohydrates are present in large quantities, in the form of cereal grains. Gradual introduction to these feeds should enable both microbial and rumen mucosal adaptation, so that VFA are readily absorbed, preventing excessively low rumen pH. However, research suggests the prevalence of SARA is a global phenomenon and maybe far more widespread across pastoral systems than previously anticipated. A study of the pH of ruminal fluid from 144 grazing mid-lactation dairy cows across 12 herds in Ireland reported 11 % of animals showed symptoms of SARA (pH ≤ 5.5), 42 % were marginally affected (pH 5.6 - 5.8), and only 47 % of animals experienced normal pH (pH > 5.8) (O'Grady et al., 2008). Similarly, Bramley et al. (2008) reported 10% of cows in New South Wales had SARA (pH 5.5-5.8) in early lactation (< 100 days in milk; DIM) when grazing herbage and supplemented with a small amount of cereal grain (2 kg at milking). The purpose of a transitioning period to either cereal grain or FB is to increase the absorptive capacity of VFA by the rumen wall and prevent the decline of ruminal pH. However, formal evaluation of the efficacy of FB transitioning methods for preventing RA or SARA in early-lactation dairy cows that are fed FB is still required, despite widespread use of transitioning techniques across NZ.

The stage of lactation during FB feeding may also impact the risk of SARA. The incidence of RA is positively correlated to increased concentrate feeding and reduced milk production (Gröhn and Bruss, 1990). Despite the assumed association between acidosis and dietary transition to a high energy diet postpartum, in agreement with Dohme et al. (2008),

Penner et al. (2007) found no correlation between a longer transitioning phase (beginning prepartum) and reduced incidence of ruminal acidosis during early-lactation. The incidence of SARA has also been correlated with parity, as primiparous heifers experience longer episodes (66 min/d longer) of sub-optimal rumen pH (< 6.0) postpartum than multiparous counterparts (Humer et al., 2015), a result supported by others (Krause and Oetzel, 2006; Bramley et al., 2008). Heifers may also spend less time chewing digesta, which reduces the buffering capacity from saliva (Humer et al., 2015). Therefore, wintering primiparous dairy heifers on a FB diet may increase their acute and SARA risk compared with multiparous dairy cows. Krause and Oetzel (2006) reported that the risk of acidosis in TMR systems might continue to increase for as long as the first three months of lactation. The observation that the risk of ruminal acidosis increases with an animal's plane of nutrition may suggest that dairy cows (lactating or non-lactating) are more likely to experience RA than beef cattle. Furthermore, early lactation dairy cows may again be at greater risk, than late lactation dairy cows fed a FB and pasture diet.

### 2.4.2 Mineral imbalance

The mineral composition of FB is characterised by low concentrations of phosphorous, calcium, and magnesium although, the sodium content is greater than pasture (Winzer et al., 1996; Waghorn et al., 2019). Animal phosphorous reserves are stored mainly in skeletal form as hydroxyapatite in a 1:1.7 ratio with calcium. Thus, bone levels of P and Ca not only reflect strength but are a reservoir for metabolism. Mechanisms regulating Ca and P homeostasis of early lactation dairy cows have been reviewed in detail elsewhere, and we refer the reader to the likes of (Horst, 1986) for greater detail.

Hypophosphatemia or ‘creeper cow syndrome’ (alert but unable to stand) can occur following parturition and contributes to the depletion (or reduced absorption) of other minerals such as Ca (causing milk-fever or hypocalcemia) and magnesium (causing grass staggers-hypomagnesemia). Hypophosphatemia is also linked to the decline of vitamin D availability experienced during winter when daylight and sunshine hours are limited (Horst, 1986). While the availability of P will vary with region and soil type, supplementation of FB with a P based mineral lick or slurry (commonly di-calcium phosphate: DCP) is recommended (Gibbs, 2014; DairyNZ, 2017). Correct supplementation of P should prevent hypophosphatemia, focusing on ensuring all cows have controlled access to mineral supplements. Caution on the use of P-based mineral licks is needed, as intake can be challenging to control and formulas will contain additional minerals such as Ca, of which less

than 50 g/d should be consumed to reduce the occurrence of milk fever postpartum (Horst, 1986). Direct application of DCP to straw or on FB before allocation will improve distribution and access of mineral supplements to all herd individuals (Gibbs, 2014).

The low P content of FB is also associated with skeletal deformities and nutritional rickets which was diagnosed in 6% of newborn lambs in response to wintering maternal ewes on FB prepartum (Dittmer et al., 2017). The low mineral and CP content of FB bulb has increased concern for *in utero* development of dairy heifers. However, carcass characteristics of calves whose mothers were fed maize silage and moderate (60% intake) levels of FB *in utero*, were normal (Moonsan et al., 2018) although the effect of appetite FB intake on skeletal characteristics of dairy heifers are unknown. It is important to note that mineral deficiencies of FB should not affect animal health when fed during lactation as content in pasture should be sufficient if FB is less than 30-40 % of DMI (Dalley et al., 2019; Waghorn et al., 2019). While the mineral deficiencies of FB are likely mitigated by correct management of prepartum mineral supplements, they still represent an inherent risk to animal health and welfare due to the relatively limited capacity for individualised feeding of mineral supplements (Hills et al., 2015).

### 2.4.3 Ketosis and hepatic lipidosis

Body condition score has become a key performance indicator for livestock production systems as an inexpensive and repeatable measure of fat reserves and animal welfare pre and postpartum. The size of adipocyte is correlated to BCS and lipolytic activity of insulin-resistant adipocytes (subcutaneous and omental) and in addition to depressed appetite, may contribute to excess mobilization (negative energy balance: NEB) during early-lactation (Morrow, 1976; Drackley, 1999; De Koster et al., 2016). Mobilization of adipose tissue increases the circulation of non-esterified fatty acids (NEFA) which accumulate as triacylglyceride (TAG) in the liver. When lipid uptake exceeds hepatic oxidation and secretion, accumulating TAG cause hepatic lipidosis (fatty liver) (Bobe et al., 2004). Incomplete oxidation of NEFA can increase the circulation of ketone bodies such as β-hydroxybutyrate (BHB) leading to hyperketonaemia and clinical ketosis (Drackley, 1999; Bobe et al., 2004; Mann et al., 2016; Phyn et al., 2017). Assuming the ME value of FB and straw are 12.0 and 9.0 MJ ME/kg DM, respectively, the total ME intake of a FB and straw diet (70:30) will be between 147 to 171 MJ ME/d (either 10 or 12 kg DM FB, respectively) which may cause excessive liveweight gain if maintained throughout the winter-dry period. Excessive liveweight gain of dairy cows

prepartum is associated with greater postpartum negative energy balance, BCS loss, risk of metabolic disorders, and postpartum anoestrous interval (Roche et al., 2004a; Roche, 2007) which reduce milk production and fertility (Drackley, 1999; Duffield et al., 2009). Nevertheless, the ability to limit ME intake, meet animal nutrient requirements, and control BCS on a winter FB diet is limited (Gibbs, 2011). It is generally accepted that cows should be removed from FB before calving based on the expected calving date and mammary tissue development. Roche et al. (2004b) concluded that cows should not be fed more than 0.93 MJ ME/kg $LW^{0.75}$ or 90.8 MJ ME/d (450 kg cow) in the final three weeks of gestation, to encourage intake and to improve postpartum NEB. Metabolizable energy requirements highlight the importance of reducing ME intake during the final weeks of gestation. The transition of animals to a high intake of FB followed by reduction of FB allocation may reduce the development of RA, due to 'gorging'. However, it is not clear whether such diets maintain rumen function and whether this risk outweighs the risk of feeding an imbalanced diet (in terms of protein, minerals, and fibre) or causes excessive liveweight gain and increased risk of metabolic disease (i.e. hepatic lipidosis or ketosis), postpartum.

The limited evidence suggests that feeding a high sugar low mineral diet such as FB may increase the risk of metabolic illness in lactating and non-lactating dairy cows. In addition, the risk of RA and SARA appears to increase with physiological state and energy requirements. Of greatest concern is the paucity of information that categorises RA and SARA incidence, ketosis, hepatic lipidosis, and mineral imbalance by region or feeding practices. While the issue of mineral imbalance and hepatic lipidosis of dairy cows that graze FB over winter will not be addressed in the current review, there is sufficient evidence to warrant concern for the welfare of dairy cows fed FB at any physiological state.

### 2.4.4 Feeding Management

One approach to improving the milk response and reducing the risk of SARA from FB could involve developing alternative feeding management. The physical and metabolic interactions controlling voluntary intake result from metabolic feedback that regulate ingestive and digestive processes (Forbes and Provenza, 2000; Forbes, 2007a). The theory of post-ingestive feedback is supported by the decline of intake and malaise in response to SARA and RA as low pH, increased osmolarity, and VFA concentration signal the cow to cease eating and develop specific aversions and display preferential behaviour towards certain feeds (Illius and Jessop, 1996; Gregorini et al., 2015). Ruminants are crepuscular grazers, consuming most of their

nutrition through one large grazing bout at dusk, with a large but secondary bout at dawn (Orr et al., 2001). The dusk grazing event may have evolved as an anti-predation response or as a behavioural adaptation to maximise nutrient intake (Gregorini, 2012). The proportion of WSC in pasture can increase from 9 to 14% between dawn and dusk, which dilutes protein, NDF, reducing tensile strength and the energy expended ingesting and digesting plant material (Orr et al., 2001; Gregorini et al., 2006; Gregorini et al., 2008; Gregorini, 2012). While allocating herbage at dusk rather than dawn may improve nutrient acquisition, the effect of time and amount of FB allocation on intake, milk production, and the risk of RA is not clear.

While herbage allowance is positively correlated to milk production and negatively correlated to milk response, increased herbage allocation frequency does not translate to increased intake (Allden and McDWhittaker, 1970; Stobbs, 1977; Dalley et al., 2001b). Allocation of pasture once daily or in six equal portions between 06:00 h and 20:00 h did not increase DMI (15.6 v. 15.9 kg DM/cow/day), grazing time (9.4 v. 9.5 h/day) or milk production (25.4 v. 25.2 L/cow/day) (Dalley et al., 2001b). Similar results are observed when the duration between meals increases, as the allocation of pasture once every four days did not alter DMI compared with cows offered pasture once daily (Abrahamse et al., 2008). However, the increased frequency of supplement allocation reduces the risk of acidosis or sub-optimal rumen pH (Kaufmann, 1976; Cohen et al., 2006). We hypothesise that reducing the meal size and increasing the frequency that FB is fed from once to twice daily will improve rumen pH when FB is used to supplement ryegrass during lactation. However, the degree that feeding frequency may improve acidosis risk or the effect on grazing behaviour, total intake, and nutrient supply of lactating dairy cows is currently unknown.

## 2.5 Integration of FB at the farm system level

### 2.5.1 Profit margin

Fodder beet is expensive to grow ($2,000-3,000/ha, NZD 1 NZ$ = 0.66 US$) compared with alternative winter forages crops such as kale ($1,000-1,400/ha) or swedes (*Brassica napobrassica*; $800-1,000/ha) (Agricom, 2018) and high yields must be achieved to justify the cost of FB/kg DM. Fodder beet yields are influenced by several climatic, agronomic (fertilisers, herbicides, and pesticides), and soil-based (fertility, structure) conditions. Although yields of > 30 t DM/ha of FB have been reported anecdotally, formal reports are much more conservative ~ 23 t DM/ha (Chakwizira et al., 2013; Chakwizira et al., 2014; Chakwizira et al., 2016), which may reflect the heterogeneity of crop yield and the need for large scale and

representative sampling for yield estimates (Gibbs et al., 2015). However, the variation of FB yield across the paddock causes inconsistent FB allocations, an additional risk factor of RA when cows graze FB.

The cost of forage crops will compound relative to the time a paddock is closed for grazing. Fodder beet requires 11-14 months, for spring cultivation, sowing, grazing/harvesting, and re-grassing (as part of a pasture renewal program). The above must occur between autumn and the following spring to minimise the time in which ground is fallow and unproductive, assuming that conditions are suitable for heavy machinery needed for harvesting or grazing stock in early/mid-spring. The cost of harvesting depends on contractor, price, availability, and distance between crop and storage area, and can vary between $300-2000/ha ($NZ), adding considerably to initial growing costs. The additional lifting costs, high cost of lost opportunity from lost milk production, variable crop yield, low milk response, and the increased risk to animal welfare will collectively increase the financial risk when FB is grown to support lactation. In comparison, maize may be sown for silage in October and harvested ~ 150 days later and pasture re-sown before winter. A crop of maize silage will yield between 18-26 t of DM/ha of the crop being grown in addition to ~ 4-7 t DM/ha of pasture (Fausett et al., 2015). However, removing pasture from the grazing rotation for at least 11 months will reduce pasture contribution to total DM yield. Based on these figures, FB yield must exceed 22 t DM/ha to be equally as productive as maize. While such yields of FB are achievable in specific locations and under irrigation, alternative options to support lactation may provide more consistent yields at reduced cost and risk to animal health.

### 2.5.2 Risk

Greater rewards are an assumed benefit of risk yet; the decision to grow FB may increase the risk for minimal return. The inclusion of any forage crop to complement pasture growth will increase the stocking rate on the platform and reduce the system's flexibility when exposed to adverse operating environments (Penno et al., 1996). Rotz et al. (2005) reported growing maize for silage increased DM yield, profit, and reduced volatile nitrogen loss of the system by 20 %, but crop yield variability significantly influenced operating costs and risk. While the inclusion of forage crops onto the milking platform can increase herbage accumulation by 31 %, the subsequent 3% increase of return on asset (**ROA**) was more conservative (Rawnsley et al., 2013). However, (Shadbolt, 2012) concluded that the ROA change in response to the purchase of supplemental feed was limited in NZ by the deregulated economy and the

subsequent fluctuation of commodity markets. A Taranaki based farm study incorporated a mixture of maize (for autumn maize silage), summer grazed turnips (*Brassica rapa var. rapa L.*), and winter grazed italian ryegrass (*Lolium multiflorum, L.*) with oats (*Avena sativa L.*), onto the milking area (De Ruiter et al., 2010). Compared with a herbage only system, the average yield of DM/ha was only 0.6 t DM greater, and as operating costs also increased due to cropping, operating profit was reduced by crop inclusion ($3,656 versus $2,655/ha, $NZ). However, the financial advantage of growing FB will depend not only on the variation of DM yield but also on the extent and the purpose for growing FB on the milking platform for either supporting early or late-lactation milk production or feeding non-lactating dairy cows over winter.

Capacity for FB crop to improve DM supply above pasture may be of greater consequence to profitability than the degree of feed deficit. Macdonald and Penno (1998) concluded that six factors determined profitability from imported or farm-grown supplements; the size of feed deficit, quality of the supplement, quality of pasture, pasture substitution rate, stage of lactation, and animal liveweight gain. However, a late-summer drought leading to lower crop yields (less than pasture) reported in De Ruiter et al. (2010), prevented crop inclusion from increasing lactation length. Furthermore, while the following year led to greater crop yield, this was not reflected by greater production level, suggesting the high-quality forages were utilised less efficiently than pasture. The results of Ramsbottom et al. (2015) support this, as a linear increase of milk yield in response to supplement, reduced profitability (- $136/ha, $NZ) and the quantity of pasture harvested (- 0.6 t DM/ha), which reflect the lower and less variable operating costs and greater pasture utilization of low-input systems (Shadbolt et al., 2017). Thus, emphasizing the need for FB to achieve high yields when fed during lactation as this must outweigh the low apparent milk response and increased operational cost of the system. Surprisingly, a formal analysis of the profitability of growing FB to support lactation is unknown.

Careful consideration of the methods used and the level of FB incorporation on the milking platform is needed. Factors that will determine whether FB is a viable option for on-farm forage will depend on whether the risk of increased stocking rate can be justified and easily mitigated under unfavourable operating environments (climate and market value of supplement feed and milk product). A combination of autumn grazing of FB and lower stocking rate may increase farm profitability and reduce risk exposure, due to a reduction of variable and fixed expenses. However, as the previous section indicates, the inclusion of FB in the diet

may be associated with increased risk of impaired animal health. Profit analysis at the whole-farm-scale is required, and evaluation of the effect on risk exposure is needed to enable dairy producers to make informed decisions.

### 2.5.3 Environmental Outcomes

The large yields achievable from FB may intensify winter grazing systems and increase the environmental impacts of agriculture. Urine patches and the inappropriate use of N fertilisers can cause very high N loading rates that far exceed plant requirements. The excess nitrogen in urea is first converted to ammonium ($NH_4^+$) by ammonia-oxidizing bacteria and then to nitrate ($NO_3^-$), which is at high risk of leaching through the soil profile into residing waterways (Cameron et al., 2013). Denitrification of $NO_3^-$ results in the volatilization of atmospheric nitrous oxide ($N_2O$), a contributor to ozone layer depletion, acidification, and eutrophication of waterways (Cameron et al., 2013). Agriculture contributed up to 48 % of NZ net GHG emission in 2017, of which 71 % is from methane, and 22 % is from $N_2O$ (MFE, 2017). While FB contains little CP (< 10 %) compared with kale (>12 %) the N concentration of urine from cows fed either FB, early, or late sown kale were similar (2.1, 2.7, 2.1 g N/L) and suggest care is needed when using FB to reduce N excretion as consideration of animal N requirements is needed (Edwards et al., 2014b). Urinary N concentrations of cows supplemented with FB (25% of daily intake) were similar to those supplemented with the same maize silage amount during late lactation (Dalley et al., 2019). While increasing the allocation of FB to 40 % of daily intake reduced urinary N concentrations further, the diet reduced milk production compared with the 25 % FB diet, presumably due to SARA (Dalley et al., 2019). In contrast, $N_2O$ emissions in urine collected from cows fed FB declined 39 % compared with urine collected from cows fed kale, despite a similar urine-N application rate (300 kg N/ha) (Di et al., 2016). Secondary plant metabolites present in either root exudate or the urine of cows fed FB can delay ammonia-oxidising bacteria's growth, slowing the oxidation of ammonia in the soil (Yao et al., 2018). However, the extent that delayed oxidation may reduce *in situ* emissions of $N_2O$ is still unclear and analysis of system-level impacts is needed, given the reduced area, and greater stocking rates winter FB systems versus other forages such as kale.

Fodder beet is advertised as a forage that reduces nitrate leaching and greenhouse gas emissions from agriculture, despite limited information which supports this claim. While FB contains low amounts of N and can reduce the N concentration of urine patches, further consideration of stocking density and the dose-dependent response to FB supplementation

suggest large quantities of FB may be required to improve environmental outcomes. Furthermore, the intake of FB required to produce a measurable response to FB may impair welfare due to nutritional imbalances such as SARA and protein deficiency.

## 2.6 Conclusion

There appears to be little advantage to supplementing pasture with FB to support lactation in NZ dairy systems. Despite widespread use in NZ, there is limited research on changes to milk production. Although, information available suggests the milk response is low compared with alternative supplements. Feeding FB in pastoral systems will also increase the risk of adverse animal health, particularly, RA and SARA. The high sugar content of FB coupled with the low content of NDF, may not complement a highly digestible pasture which is also low in NDF. Factors that contribute to SARA risk include variability among individuals, large herd sizes, animal physiological state and stage of lactation, and difficulty diagnosing affected animals. While FB has been utilised internationally for centuries, grazing and feeding harvested bulb as is practised in NZ is novel and reduces FB intake control. Repeated research is required to ascertain the risks at different lactation stages and the possibility of feeding an imbalanced diet prepartum on postpartum metabolic disease and fertility. High and consistent FB yields are needed to maintain profitability, although consideration of animal welfare, economic and environmental outcomes suggest alternative options should be considered if possible.

# Chapter 3

# Milk production and milk fatty acid composition of grazing dairy cows supplemented with fodder beet

*Published in the New Zealand Journal of Animal Science and Production 2018 (78:6-10)*

## 3.1 Abstract

A study was conducted to evaluate the effect of supplementing a perennial ryegrass-based diet with fodder beet on milk production and milk fatty acid composition, of dairy cows in early lactation. Sixty Friesian × Jersey cows were blocked into six groups of 10 cows, and groups randomly allocated to three replicates fed either 18 kg DM/day of ryegrass herbage (H), or 14.4 kg DM/day of ryegrass herbage + 4 kg DM of harvested FB bulbs (FBB). Dry matter intake (DMI) was similar between H and FBB (15.0 ± 0.77 and 14.2 ± 0.48 kg DM/day, respectively). Although milk yield tended to be greater for H than FBB (20.0 and 18.9 kg/day, respectively; $P$ = 0.09); milk solids production was not affected by treatment ($P$ = 0.89). Supplementation with FBB increased the saturated (80.6 versus 73.2 ± 0.39 g/100g FA; $P$ < 0.001) and medium-chain milk fatty acid (66.7 versus 56.2 ± 0.783 g/100g FA; $P$ < 0.001) content, compared with H. Under the conditions of the present study, our results suggest that supplementing grazing dairy cows with FBB in early lactation, may not improve milk production and increases the saturated FA content of milk.

## 3.2 Introduction

The large crop yields achieved by fodder beet, *Beta vulgaris L.,* (FB) have led to its extensive use as a winter forage crop in New Zealand dairy systems. Compared with an alternative forage such as kale ( *Brassica oleracea*) which yields between 12-16 t DM/ha, FB can produce >20 t DM/ha (Chakwizira et al., 2013), which can be grazed or harvested and fed elsewhere or stored if necessary. This versatility is attractive for many farmers, as FB may be harvested to return the land to pasture and the FB fed to supplement the early lactation herbage supply. Although FB bulbs are high in metabolisable energy [ME: 11.8 MJ ME/kg DM (Clark et al., 1987)], studies undertaken internationally report minimal improvement of milk yield when FB is fed alongside various levels of protein (Fisher et al., 1994), or concentrates (Ferris et al.,

2003). However, the milk response to supplementing a grazed herbage diet with FB has had little study.

Human food production from agriculture has traditionally focused on quantity. However, consumers are becoming increasingly aware of the quality and associated health risks or rewards. For example, the concentration of poly-unsaturated fatty acids (PUFA) such as conjugated linoleic acid (CLA: C18:2 *cis*-9, *cis*-12) or α-linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15), have anti-carcinogenic properties and are associated with a range of benefits related to human health (Chilliard et al., 2000). Conversely, saturated fatty acids (SFA), have been linked with increased plasma concentrations of low-density lipoprotein cholesterol, which may be a risk factor for cardiovascular disease (Shingfield et al., 2013). While herbage contains high concentrations of C18:3, and to a lesser extent CLA, ruminal biohydrogenation reduces their abundance in milk (Chilliard et al., 2000). Supplementation with starch can increase the PUFA content of milk as a result of reduced bio-hydrogenation at lower rumen pH (Kolver and De Veth, 2002). While FB contains little starch, it is rich in soluble sugars, principally sucrose (Clark et al., 1987), which may also lower ruminal pH and increase the PUFA content of milk. Consequently, the objective of this research was to determine the effect of substituting the herbage of grazing dairy cows with FB on milk production and milk fatty acid (FA) composition. We hypothesised that supplementing lactating dairy cows with FB would improve milk production and increased the PUFA content of milk compared with cows fed herbage only.

## 3.3 Methods

### 3.3.1 Experimental site and design

All animal treatments and measurements in this experiment were approved by the Lincoln University Animal Ethics Committee (#2016-30).

The experiment was conducted between the 10$^{th}$ and 25$^{th}$ of November 2016 at Lincoln University's Ashley Dene Research and Development Station in Canterbury (-43.65 º North, 172.33 º East), New Zealand. Sixty Friesian x Jersey (F9 J6) dairy cows were blocked into three replicate groups according to live weight (438 ± 3.1), age (3.6 ± 0.12 years) days in milk (DIM: 85 ± 4.8 days), and milk solids (MS: 2.02 ± 0.18 kg/day) and allocated to two treatments in a completely randomised design. Treatments were: perennial ryegrass (RG: *Lolium perenne* L.*)* and white clover (WC: *Trifolium repense*) sward, offered as a herbage only diet (H); or herbage + 4 kg DM/day of harvested FB bulb (FBB).

### 3.3.2 Grazing management

Fodder beet (cv. Rivage) was sown in October 2015, harvested commercially and stored five weeks prior to the study. Perennial ryegrass and white clover swards were grazed 4 ± 1 weeks prior to the experiment and fertilised with 46 kg N/ha as urea. Prior to the experiment, all cows grazed a PRG WC sward supplemented with 3.5 kg DM/d of harvested FBB. An eight-day transition period prior to the experiment enabled animals in H to adapt back to a herbage only diet, and the FBB cows to reach FB allocation.

Over the measurement period, herbage DM allocation for cows offered either H or FBB were 18 and 14.4 kg DM/cow per day, respectively above a residual herbage compressed height (as determined by rising plate meter, RPM) of 3.5 cm or 1500 kg DM/ha. Access to water was *ad libitum*. Cows were offered a fresh allocation of herbage each morning behind a temporary electric fence with a back fence to prevent grazing of residual regrowth. Allocation area was calculated from herbage mass estimated by RPM (Jenquip Ltd, New Zealand); using a standard equation for PRG WC swards (kg DM/ha = 140 x RPM reading + 500). Replicate groups of FBB grazed a single paddock split into three breaks, while two groups in H shared a paddock and the remaining group grazed alone. Prior to the experiment, the DM (20.3%) of FBB was determined by a random selection of bulbs in the stack, and oven drying (60°C for 48 hours). Daily DM allocations of FBB were weighed and fed out by mixer wagon onto a feed pad and fed to cows after the morning milking. Upon meal completion, cows were returned to pasture. Animals were milked at 0700 and 1600 h daily.

### 3.3.3 Feed measurements and analyses

Herbage was sampled on four occasions, before and after grazing, by plucking ten random hand grab samples to grazing height in each allocation. Herbage was bulked and sub-sampled to assess DM (oven-dried at 60° C for 48 hours), botanical and chemical composition. Samples of FBB were also collected, minced and stored at -20ºC. Supplement refusals were collected daily and weighed to determine apparent intake. Sub-samples of pasture were freeze-dried then ground (ZM200 Retsch) for chemical analysis (FA, crude protein: CP, acid detergent fibre: ADF, neutral detergent fibre: NDF, organic matter: OM, and water-soluble carbohydrates: WSC) using near-infrared spectroscopy (NIRS. Model: FOSS NIRS Systems 5000, Maryland USA). Ground samples of FBB were assessed for N: Elementar (Variomax CN Analyser, Elementar Analysensysteme, Germany), ADF, NDF (Van Soest et al., 1991b), ash and WSC (Pollock and Jones, 1979).

### 3.3.4 Animal measurements and analyses

Milk yield (kg/d) and live weight were recorded automatically at each milking (Waikato milking systems, Hamilton, New Zealand). Bulk milk from individual cows was sub-sampled from two consecutive milkings on six occasions to determine milk fat, protein and lactose, using Milkoscan (Foss Electric, Hillerod, Denmark, courtesy of Livestock Improvement Corporation, Christchurch). A skimmed sample of milk was used to determine milk urea nitrogen (MUN), using a Randox RX Daytona analyser (clinical assay kit: UR 3825, Randox Rx Daytona, Randox Laboratories, Ltd, United Kingdom). Fatty acid methyl esters of milk, pasture and FB were prepared by transmethylation and analysed by gas chromatography (Shimadzu GC-2010, Japan with AOC-20i auto-sampler) using a Varian CP742 silica capillary column (0.25 x 100m x 0.2 µm), following AOAC method 2012.13 (Horwitz and Latimer, 2005).

## 3.4 Statistical Analysis

All individual animal variables were combined and averaged over sampling days and analysed by ANOVA using GenStat (v.18 VSN International LTD, 2015) with two treatments and three replicates equating to six experimental units. The treatment effect was deemed significant if $P \leq 0.05$.

## 3.5 Results

### 3.5.1 Feeds

The pre-grazing herbage mass and ME intake were similar between H and FBB groups. However, post-grazing herbage mass ADF, NDF, and reproductive pasture were greater, and CP lower in herbage fed to FBB compared with that of H (Table 3.1). Fodder beet contained more WSC and less CP, ADF and NDF than herbage (Table 3.1). While there was no variation in the FA content of herbage between treatments, FBB contained less C18:3 (0.17 versus 8.9 mg FA/g DM, $P < 0.001$) and CLA (1.29 versus 2.25 mg FA/g DM, $P < 0.001$) than herbage (Table 3.1).

### 3.5.2 Animal

Animals did not achieve target herbage residuals (1500 kg DM/ha) resulting in average apparent herbage DM intakes of 14.9 and 10.2 ± 0.625 kg DM/day for H and FBB respectively during the measurement period. When supplement was accounted for, there was no difference in total apparent DMI between FBB and H (14.2 vs 15.0 ± 0.63 kg DM/d; $P = 0.4$)

respectively. While live weight declined in both treatments over the experimental period, this was more pronounced in FBB groups than H (-9.93 vs -4.95 kg). Animals offered FBB tended ($P$ = 0.09) to produce less milk, but the MS yield was not different from those measured in the H groups (Table 3.2). Lactose yield (0.98 versus 1.04 ± 0.03 kg/day, $P$ = 0.02) and MUN (3.48 vs 6.58 mg/dl $P$ < 0.001) were lower and percentage fat greater for cows fed FBB; however, milk fat yield was not significantly different. Concentrations of saturated ($P$ < 0.001), short-chain (< C8, $P$ < 0.001), medium-chain FA (C8-C16, $P$ < 0.001) were higher and PUFA lower, in milk produced from FBB rather than H. There was no treatment effect on C14:1/C14:0 ($P$ = 0.99) or C16:1/C16:0 ratios ($P$ = 0.21); however, cows fed FBB had lower C18:1 to C18:0 ($P$ < 0.001) and CLA C18:2 *cis9*, *trans11* to C18:1 *trans11* ($P$ < 0.001) ratios than those fed H (Table 3.2).

Table 3.1. Pre and post-grazing herbage mass and chemical composition of herbage offered to cows grazing either a herbage only (H) or a herbage and fodder beet diet (FBB herbage). The chemical composition of fodder beet bulbs (FB bulbs) is also presented.

| Variable | H | FBB herbage | FB Bulb | SEM[1] | *P*-Value |
|---|---|---|---|---|---|
| Pre-graze mass (kg DM/ha) | 3642[a] | 3581[a] | | 170 | 0.804 |
| Post-graze mass (kg DM/ha) | 1663[a] | 2003[b] | | 57.4 | <0.001 |
| Reproductive grass (% DM) | 32.2[a] | 57.1[b] | | 3.65 | <0.001 |
| Vegetative grass (% DM) | 40.6[a] | 32.8[a] | | 5.03 | 0.111 |
| Clover (% DM) | 10.5[a] | 0.98[b] | | 1.81 | 0.001 |
| Dead (% DM) | 5.67[a] | 6.20[a] | | 1.26 | 0.745 |
| Weeds (% DM) | 11.1[a] | 5.16[a] | | 2.45 | 0.237 |
| Herbs (% DM) | 0[a] | 6.20[a] | | 0.24 | 0.329 |
| Dry Matter (%) | 22.4[a] | 26.5[b] | 20.3[c] | 1.73 | 0.033 |
| Nitrogen (%) | 2.44[a] | 1.89[b] | 1.30[c] | 0.99 | <0.001 |
| WSC[2] (%) | 24.1[a] | 26.8[b] | 54.9[c] | 0.41 | <0.001 |
| ADF[3] (%) | 25.2[a] | 28.8[b] | 6.7[c] | 1.03 | 0.021 |
| NDF[4] (%) | 41.6[a] | 45.7[b] | 14.2[c] | 0.93 | 0.005 |
| CP[5] (%) | 15.3[a] | 11.8[b] | 8.5[c] | 0.45 | <0.001 |
| OM[6] (%) | 80.8[a] | 79.5[a] | 94.7[b] | 0.10 | <0.001 |
| ME (MJ ME/kg DM) | 11.7[a] | 11.5[a] | | 0.12 | 0.132 |
| **Fatty Acid (FA) Content** | | | | | |
| C16:0 (mg/g DM) | 3.01[a] | 2.95[a] | 0.84[b] | 0.1 | <0.001 |
| C18:0 | 0.27[a] | 0.24[a] | 0.07[a] | 0.1 | 0.109 |
| C18:1 | 0.45[a] | 0.47[a] | 0.68[a] | 0.22 | 0.733 |
| C18:2 | 2.3[a] | 2.2[a] | 1.29[b] | 0.04 | <0.001 |
| C18:3 | 9.0[a] | 8.80[a] | 0.17[b] | 0.71 | 0.001 |
| Σ Total FA | 16.2[a] | 16.1[a] | 3.32[b] | 0.62 | <0.001 |

[a-c] Means of the same variable in the same row with different superscripts differ. [1] Standard error of the mean. [2] Water-soluble carbohydrates. [3] Acid detergent fibre. [4] Neutral detergent fibre. [5] Crude protein. [6] Organic matter

Table 3.2. Change in live weight, the yield of milk and milk constituents and milk fatty acid (FA) composition of cows fed either herbage only (H) or herbage and 4 kg DM of harvested fodder beet (FBB).

| | H | FBB | SEM[1] | *P*-Value |
|---|---|---|---|---|
| LW change (kg) | -5.0[a] | -9.9[b] | 0.811 | <0.001 |
| Milk (kg) | 20.0[a] | 18.9[a] | 0.419 | 0.091 |
| Fat (%) | 5.32[a] | 5.75[b] | 0.088 | <0.001 |
| Protein (%) | 3.94[a] | 4.03[a] | 0.043 | 0.155 |
| MS (%) | 9.26[a] | 9.78[b] | 0.123 | 0.004 |
| Fat (kg/d) | 1.05[a] | 1.08[a] | 0.021 | 0.301 |
| Protein (kg/d) | 0.78[a] | 0.76[a] | 0.014 | 0.217 |
| MS (kg/d) | 1.84[a] | 1.84[a] | 0.033 | 0.898 |
| Lactose (kg/d) | 1.04[a] | 0.98[b] | 0.022 | 0.036 |
| MUN mmol/L | 6.58[a] | 3.48[b] | 0.173 | <0.001 |
| **FA (g/100g FA)** | | | | |
| C4:0 | 1.34[a] | 1.41[b] | 0.00 | 0.057 |
| C6:0 | 1.48[a] | 1.56[b] | 0.00 | 0.016 |
| C8:0 | 1.13[a] | 1.22[b] | 0.00 | <0.001 |
| C10:0 | 3.14[a] | 3.81[b] | 0.10 | <0.001 |
| C12:0 | 3.81[a] | 5.03[b] | 0.10 | <0.001 |
| C14:0 | 12.4[a] | 14.0[b] | 0.30 | <0.001 |
| C16:0 | 35.8[a] | 41.9[b] | 0.40 | <0.001 |
| C16:1 c7 | 0.22[a] | 0.20[b] | 0.00 | <0.001 |
| C16:1 c9 | 1.25[a] | 1.28[a] | 0.03 | 0.403 |
| C18:0 | 9.28[a] | 7.25[b] | 0.20 | <0.001 |
| C18:1 c6 | 0.38[a] | 0.25[b] | 0.01 | <0.001 |
| C18:1 c9 | 15.1[a] | 9.80[b] | 0.23 | <0.001 |
| C18:1 t9 | 0.16[a] | 0.11[b] | 0.00 | <0.001 |
| C18:1 t11 | 2.28[a] | 1.47[b] | 0.11 | <0.001 |
| C18:1 t5 t8 | 0.15[a] | 0.10[b] | 0.00 | <0.001 |
| C18:2 c9 c12 | 0.90[a] | 0.71[b] | 0.01 | <0.001 |
| C18:2 c9 t 13 | 0.14[a] | 0.10[b] | 0.00 | <0.001 |
| C18:2 t9 c12 | 0.12[a] | 0.07[b] | 0.01 | <0.001 |
| C18:3 c9, 12, 15 | 0.93[a] | 0.69[b] | 0.02 | <0.001 |
| CLA c9 t11 | 0.87[a] | 0.50[b] | 0.03 | <0.001 |
| Σ Short chain (total FA) | 7.26[a] | 8.37[b] | 0.12 | <0.001 |
| Σ Med chain FA | 56.2[a] | 66.7[b] | 0.78 | <0.001 |
| Σ Long chain FA | 34.8[a] | 24.9[b] | 0.58 | <0.001 |
| Σ SFA[2] | 73.2[a] | 80.6[b] | 0.39 | <0.001 |
| Σ Mono FA | 22.6[a] | 16.4[b] | 0.51 | <0.001 |
| Σ PUFA[3] | 3.8[a] | 2.86[b] | 0.06 | <0.001 |
| **Product: substrate ratios** | | | | |
| C14:1 *cis 9* to C14:0 | 0.06[a] | 0.06[a] | 0.00 | 0.987 |
| C16:1 *cis 9* to C16:0 | 0.03[a] | 0.03[a] | 0.00 | 0.210 |
| C18:1 *cis9* to C18:0 | 1.64[a] | 1.36[b] | 0.03 | <0.001 |
| CLA C18:2 *cis9, trans11* to C18:1 *trans 11* | 0.36[a] | 0.34[b] | 0.01 | <0.001 |

[a-b]Means of the same variable in the same row with different superscripts differ

[1] Standard error of the mean. [2] Saturated fatty acid. [3] Poly-unsaturated fatty acid

## 3.1 Discussion

There was a tendency ($P$ = 0.09) for cows to produce less milk when fed FBB, however, due to the greater proportion of solids in milk (Table 3.2), MS production was similar across treatments. The higher solids in FBB milk reflects a greater percentage of milk fat (5.75 versus 5.32%), similar to previous reports (Fisher et al., 1994; Ferris et al., 2003). The advanced phenological state of herbage (Table 3.1) led to a relatively low CP content of both diets, below the 18% recommended level for early lactation dairy cows (14.5 and 15 % CP FBB and H) respectively. The animal response to this change in nutrient supply altered milk composition. For example, MUN was lower for cows fed FBB compared with those in the H treatment (3.58 and 6.58 mmol/L FBB and H) respectively

The lower lactose concentration and yield in FBB milk (Table 3.2), suggests a limited supply of glucogenic precursors (propionate or glucogenic amino acids), and that animals in FBB were in a state of negative energy balance (NEB). This is supported by the greater reduction of live weight of FBB cows (Table 3.2). However, NEB did not affect the FA content of milk, as the proportions of C18 FA in milk will increase when circulatory concentrations of lipoproteins and non-esterified fatty acids (indicative of NEB) are elevated (Table 3.2) (Chilliard et al., 2000).

The inclusion of FBB reduced the nutraceutical properties of 'pasture-based milk' by increasing the content of SFA: C12:0, C14:0, and C16:0 by 32, 13, and 17 % respectively, compared with H. Similarly, Collomb et al. (2004) reported increased content of C16:0 when hay was supplemented with FB, compared with rapeseed or linseed (31.1, 26.2 and 24.5 g/100g FA respectively). Fatty acids less than 12 carbons in length, most C14 and about half of C16, are synthesised *de novo* in mammary tissue, from acetate and β-hydroxybutyrate derived from rumen fermentation. Alternatively, the remaining C14, C16, and all FA longer than C18 enter the mammary gland from the arterial circulation (Chilliard et al., 2000). Incomplete biohydrogenation of unsaturated FA (UFA) reduces *de novo* synthesis in the mammary gland (Chilliard et al., 2000; Shingfield et al., 2013). Thus, in the present study, the lower UFA content of FBB (Table 3.1), could have increased *de novo* synthesised fatty acids found in milk. Furthermore, ruminal fermentation of sucrose is reported to favour butyrate

production and may increase hydrogenation of UFA (Oba, 2011), which could explain the greater milk fat percentage observed from animals fed FBB.

Unsaturated fatty acids are also synthesised in mammary tissue by delta-9-desaturase. Although herbage contains less *cis*-9, *cis*-11 CLA and more C18:3 FA than grain, the concentration of *cis*-9, *trans*-11 CLA in milk, is greater from cows fed herbage (Chilliard et al., 2000). In the biohydrogenation pathway of linoleic acid, *cis*-9, *cis*-12 C18:2 is isomerised to cis-9, trans-11 CLA, yielding C18:1 *trans*-11 (vaccenic acid) and finally, C18:0. While hydrogenation of linolenic acid also yields vaccenic acid, the formation of *cis*-9, *tr*ans-11 CLA is not an intermediary step. The increase of *cis*-9, *tr*ans-11 CLA in pasture-based milk suggests a proportion of *cis*-9, *trans*-11 CLA is formed through desaturation of *tra*ns-11 C18:1, by delta-9-desaturase (Griinari and Bauman, 1999). In the present study, the ratios of *cis*-9, *tr*ans-11 CLA to *trans*-11 C18:1, and *cis*-9 C18:1 to C18:0 were reduced by FBB treatment (Table 3.2); indicating a reduction of endogenously synthesised unsaturated FA. The decline of product: substrate ratios reflects the lower supply of *trans*-11 C18:1 in FBB (Table 3.1), as the activity of delta-9-desaturase is dependent on substrate availability (Kay et al., 2002). It is important to note that NEB can also inhibit delta-9-desaturase (Kgwatalala et al., 2009), and may also be partly responsible.

While FBB reduced the CLA *cis*-9, *trans*-11 and C18:3 content of milk (Table 3.2), the levels observed in H groups (0.87 CLA and 0.93 C18:3 g/100g FA) were much less than those reported elsewhere (1.81 CLA and 1.64 C18:3 g/100g) (Rugoho et al., 2014). The majority of FA synthesis occurs in chloroplasts, containing more than half of plant protein (Rugoho et al., 2017). Therefore, the generally low CP content of herbage fed presently (< 16% CP; Table 3.1), may have corresponded to lower PUFA content of pasture, and subsequently reduced the CLA and C18:3 content of milk.

Our results do not support the hypothesis that rapidly fermentable carbohydrates in FBB increase the PUFA content of milk. The appearance of dietary PUFA in milk results from ruminal passage and escape of hydrogenation by rumen bacteria (Chilliard et al., 2000). While a low rumen pH reportedly reduces hydrogenation (Kolver and De Veth, 2002), the greater proportion of fibre in FBB herbage (Table 3.1), may have increased rumen retention and biohydrogenation. In addition, the high reproductive content of herbage in FBB swards may

have contributed to lower apparent herbage intake, further limiting ruminal PUFA supply to the FBB treatment.

## 3.2 Conclusions

Our findings indicate that supplementing grazing dairy cows with FBB was of no advantage to yield, or the nutritional value of milk. The extent of ruminal bio-hydrogenation and milk FA synthesis appeared to increase, while the dietary supply of PUFA declined with FBB inclusion. However, we cannot discern whether this result was independent of the variation in herbage composition across treatments; thus, further study is required.

# Chapter 4

# Supplementation of spring pasture with harvested fodder beet bulb alters rumen fermentation and increases the risk of sub-acute ruminal acidosis during early lactation

*Published in Animals (August 2020)*

## 4.1 Simple Summary

Fodder beet (FB) is used widely in pastoral dairy systems of New Zealand to support early- and late-lactation milk production, however, the large fraction of water-soluble carbohydrate present in FB bulbs present a risk of sub-acute and acute ruminal acidosis. Despite the widespread use of FB across NZ, the incidence of ruminal acidosis using industry-recommended methods of feeding FB has not been investigated. This study analysed the time-dependent changes to rumen fermentation, apparent dry matter intake, milk production, milk composition and plasma amino acid concentration of grazing dairy cows supplemented with moderate amounts (40% of dry matter intake) of FB during early-lactation. Our findings indicate that the incidence of sub-acute ruminal acidosis due to FB is greater than currently realised as 1/4 of cows developed severe sub-acute ruminal acidosis following the transition to target FB allocation (40% of daily intake). Across all cows, FB reduced ruminal pH, feed conversion efficiency and was not advantageous to milk production. These results suggest methods for adapting cows to a diet containing FB require further evaluation to reduce the risk of SARA experienced by individuals within the herd.

## 4.2 Abstract

In a cross-over design, eight rumen cannulated dairy cows were used to explore the industry recommended method for the transition to fodder beet (FB: *Beta vulgaris* L.) on changes to rumen fermentation and pH, milk production, dry matter intake (DMI) and the risk of subacute ruminal acidosis (SARA), during early lactation. Cows were split into two groups and individually allocated a ryegrass (*Lolium Perenne* L.) and white clover (*Trifolium repens* L.) diet (HO) or the same herbage supplemented with 6 kg DM/cow of harvested fodder beet bulb

(FBH). Dietary adaptation occurred over 20 days consisting of: stage 1: gradual transition to target FB intake (days 1-12, + 0.5 kg DM of FB/d); stage 2: acclimatisation (days 13-17) and stage 3: post-adaption sampling (days 18-20). Response variables were analysed as a factorial arrangement of diet and stage of adaption using a combination of ANOVA and generalised linear mixed modelling. Dietary proportion of FB represented 22, (stage 1), 32 (stage 2) and 38% (stage 3) of daily DMI. One cow during each period developed SARA from FB, and the duration of low pH increased with the FBH treatment ($P < 0.01$). Rumen concentrations of lactate and butyrate increased with FBH, but concentrations of acetate, propionate and the total volatile fatty acids (VFA) concentration declined 9.3% at day 20, compared with the HO treatment ($P < 0.01$). Treatments did not affect milk production but estimated DMI with supplemented cows increased during the final stage of adaptation and feed conversion efficiency (FCE kg milk/kg DM) declined with the FBH treatment. The occurrence of SARA in 25% of animals fed FB suggest it is a high-risk supplement to animal health and further evaluation of industry recommended methods for feeding FB at the individual- and herd-scale are needed.

## 4.3 Introduction

New Zealand dairy farms have come to rely on fodder beet to increase body condition score (BCS) within a short 6-8-week timeframe over the winter dry cow period. The potential to obtain large yields (> 20 t dry matter: DM/ha), of high metabolisable energy bulb (~12 MJ ME) that is ~90% utilised when grazed in situ, drives the popularity of FB in NZ systems (Clark et al., 1987; Chakwizira et al., 2013; Saldias and Gibbs, 2016). The versatility to either graze *in situ* or harvest FB bulb has led to systems which graze FB during late lactation and harvest residual winter FB to supplement the postpartum herbage supply, in spring. Previous research suggests there is little advantage to milk production when feeding FB to grazing dairy cows (Chapter 3)(Dalley et al., 2019; Pacheco et al., 2020), indicating increased substitution rate (kg DM herbage/kg DM FB) and reduced milk response to FB supplement (kg milk/kg DM supplement), compared with alternative feeds (Stockdale, 2000; Bargo et al., 2003). The low milk response to FB may also indicate impaired rumen function, as FB bulb contains small amounts of fibre (< 20 %) and crude protein (CP; < 10%) and large amounts of readily fermentable water-soluble carbohydrate (WSC; >65 %) (Dalley et al., 2017). Thus, FB may limit

the CP and fibre content of the diet and increase the risk of acute or subacute ruminal acidosis (SARA) (Gibbs, 2014; Dalley et al., 2019; Waghorn et al., 2019)

Subacute ruminal acidosis is characterised by the accumulation of volatile fatty acids (VFA), which reduce ruminal pH (Owens et al., 1998). Low ruminal pH may inhibit microbial activity and reduce the digestibility of structural carbohydrates (cellulose and hemicellulose). Low ruminal pH can also cause anorexia, reduced rumination and secretion of saliva containing phosphate and bicarbonate buffers (Nocek, 1997; Kleen et al., 2003). Exposure of the rumen wall to low pH conditions (pH<5.6) impair barrier function and can cause para-hyperkeratosis of epithelial cells, which limit animal production and welfare long-term (Steele et al., 2009; Zebeli and Metzler-Zebeli, 2012b). The keratinization of the stratum corneum (outermost cells adjacent to ruminal contents) can reduce VFA absorption and bicarbonate exchange, which is an effective mechanism for neutralizing VFA and stabilizing pH (> 50% of all VFA), and enhances the risk of SARA re-occurrence (Bull et al., 1965; Allen, 1997; Dohme et al., 2008; DeVries et al., 2009). Reduced integrity of barrier function can result in the translocation of endotoxin, present in the cell wall of gram-negative bacteria, into the portal circulation, causing inflammation-mediated liver changes and laminitis (Nagaraja et al., 1978; Nocek, 1997; Gozho et al., 2005; Zebeli et al., 2012b). Other symptoms of SARA include reduced or erratic feed intake, milk fat depression and diarrhoea (O'Grady et al., 2008). Subacute ruminal acidosis is characterised by daily episodes of low ruminal pH (Plaizier et al., 2008) and reduced buffering capacity (Owens et al., 1998; Plaizier et al., 2008), but is self-corrected. Declining pH cause the proliferation of *Lactobacilli* which produce lactate that is 10-fold the acidity of other organic acids and causes a downward spiral of ruminal pH, leading to acute systemic acidosis that the animal is unable to correct (Owens et al., 1998).

Mean ruminal pH is a poor technique for defining SARA in commercial dairy systems. While there is wide variation in response between individual animals, and limited clinical symptoms (O'Grady et al., 2008), Kleen and Cannizzo (2012) described SARA from spot-samples of rumen fluid as severe when ruminal pH is < 5.5 and marginal when ruminal pH is less than either 5.8 or 5.6, in cows on pasture-based diets. The use of other indicators such as faecal consistency, low milk production and feed conversion efficiency (FCE), feed intake (Fulton et al., 1979), increased circulation of endotoxin and acute-phase proteins, provide some aid for determining the severity of SARA, but individually are poor indicators of the

disease in grazing dairy cows (O'Grady et al., 2008; Danscher et al., 2015). Continuous monitoring of ruminal pH is less practical for commercial dairy producers but is the most accurate approach for describing SARA (Duffield et al., 2004). The duration of pH below a threshold of 5.8 (Zebeli et al., 2008) for >180 minutes is defined as marginal SARA while pH less than 5.6 for >180 minutes is defined as severe SARA (Gozho et al., 2005), due to the relationship between pH and the impairment of microbial activity and structural integrity of the rumen epithelium.

Current industry recommendations suggest non-lactating dairy cows can be safely transitioned to *ad libitum* (or appetite) amounts of FB over 14 days, by initially feeding ~2 kg DM/cow per day and increasing FB allocation by either 0.5 kg DM/cow every day or 1 kg DM/cow every second day (Gibbs, 2014; DairyNZ, 2018b). While *ad libitum* intake of FB and high ruminal pH > 6.0 have been reported for steers using the 14 d transitioning method (Prendergast and Gibbs, 2015), an animal's risk of developing SARA is defined by its physiological state. Compared with beef steers, dairy cows may be more prone to SARA with lactation and stage of lactation, increasing the level of risk (Penner et al., 2007). For example, Waghorn et al. (2018) reported five out of eight non-lactating dairy cows developed acute SARA using the 14-day transition technique among animals with a final allocation of over 80% of their diet as FB. Similarly, those authors observed acidosis in late lactation cows transitioned onto a diet of 45% and 60% FB (Waghorn et al., 2018). Further analysis suggests FB bulb should not exceed 30-40% of daily DMI during lactation, due also to the low N content of the bulb (Dalley et al., 2019; Waghorn et al., 2019; Pacheco et al., 2020). Absorption of VFA across the rumen epithelium is a primary mechanism for maintaining ruminal pH (Dijkstra et al., 2012), but morphological changes to papillae size number increase gradually (6-8 weeks) postpartum, and absorptive capacity may be less than immediately required (Penner et al., 2007). Thus, the limit of FB allocation during early lactation, which will maintain ruminal pH and protein demands requires definition.

The objective of this experiment was to measure the time-dependent changes to rumen function and fermentation and test the hypothesis that early lactation dairy cows can be safely transitioned and adapted to moderate (~40% daily intake) intake of FB, using industry-approved methods.

## 4.4 Materials and Methods

An early lactation grazing experiment was conducted between October and November of 2018 at the Lincoln University Research Dairy Farm (LURDF) Canterbury, New Zealand (43°38'S, 172°27'E). All procedures were approved by the Lincoln University Animal Ethics Committee (AEC 2018-22).

### 4.4.1 Experimental Design and Treatments

In a cross-over design, eight Holstein Friesian X Jersey dairy cows in their third lactation and fitted with a rumen cannula in February 2018, were separated into two groups based on days in milk (DIM; 29.63 ± 11.6, mean ± SD), milk yield (27.4 ± 5.25, kg/day) and liveweight (482 ± 50.0, kg). Cows were randomly assigned to one of two treatments; HO, a herbage only control, consisting of approximately 19 kg DM/cow per day, above a post-grazing height of 3.5 cm, of an established perennial ryegrass and white clover sward, or FBH, which consisted of 19 kg DM/cow per day of herbage and 6 kg DM of harvested FB bulb, CV Enermax. Response variables were analysed as a 3x2 factorial arrangement of diet and adaptation stage in a cross over design consisting of two periods and eight replications per treatment. Each adaptation period consisted of three stages; transitioning during days 1-12 (stage 1), acclimatization during days 13-17 (stage 2) and full adaptation during days 18-20 (stage 3). Transition refers to the gradual increase of FB allocation (+ 0.5 kg DM/cow/day) between days 1-12. Adaption refers to the acclimatization of intake, VFA, ruminal pH, milk fatty acids (reflect rumen biohydrogenation) and plasma amino acids, which were estimated to occur over five days (days 13-17), as previously reported for supplementation with concentrate (Rego et al., 2016). Cows were assumed to be fully adapted and achieve consistent measures of production (estimated intake of DM and FB, milk production, milk composition and milk FA profiles) from the FBH diet between days 18 and 20. Dry matter intake and milk production data from day 20 were removed from the current analyses as cows were removed from the paddock at 2200 h and fasted overnight as part of a separate experiment. Cows were milked twice daily at approximately 0700 h and 1600 h and had free access to fresh water at all times except during milking. After completing the first period, a washout phase of 5 days occurred between

periods to prevent first-order carry-over effects (Senn, 2002). After the washout period, the same process was repeated, and cows were fed the opposite dietary treatment.

### 4.4.2 Feed management

Fodder beet was sown by precision drill on the 14th of November 2017 in a Templeton silt loam soil at 90,000 seeds/ha. Fodder beet was harvested, removed of the residual leaf, and transported to the experiment site before commencement of each period to maintain the bulb's chemical composition. Fodder beet was allocated to each cow individually in plastic bins on a concrete feed pad, following the morning milking. Cows remained on the feed pad for up to two hours or until completion of the FB meal before returning to a new herbage allocation.

Individual pasture breaks were allocated daily following the morning milking, in horizontal strips across each paddock. Within the strip, each cow was individually separated using electric tape to estimate daily herbage intake of each animal. Botanical and chemical composition of the sward were determined every three days, before break allocation by collecting random hand grab samples of herbage (n = 5 per break) at grazing level (~ 3 cm above ground). On the same days, two random 0.2 $m^2$ quadrats from each allocation were harvested to ground level following the compressed pasture height measurement with a rising plate meter (RPM: Jenquip Ltd, New Zealand). An additional two quadrats were taken from each allocation post-grazing. Harvested herbage was washed to remove soil contamination, dried at 60°C in a force air oven to a constant weight to determine total DM. Dry matter yields were used to estimate herbage mass from the compressed sward height using multiple linear regression for both pre and post-grazing herbage mass in each period. Paddock, period and sward state (pre/post grazing) were significant ($P < 0.05$) and included in the final regression equations while the effect of treatment or cow was not significant and therefore were not included in the final model. The final regressions were used to determine herbage mass and estimate herbage dry matter intake:

Period 1: Post-grazing mass (kg DM/ha) = 1377.6 + (121.2 x RPM)

(4.1)

Pre-grazing mass = 1792.6 + (121.2 x RPM)

Period 2: Post-grazing mass (kg DM/ha) = 391.1 + (121.2 x RPM)

Pre-grazing mass = 848.6 + (121.2 x RPM)

$r^2$ = 0.755, $n$ = 357, $P$ < 0.0001.

Rising plate meter (RPM) is the compressed pasture height measured in 0.5 cm increments. Approximately 30 RPM readings were recorded in each allocation each day to determine herbage allocation and apparent DMI. Herbage mass was estimated using calibration equations, and daily DMI was estimated using Equation 4.2.

Estimated DMI = (Pre-grazing mass – Post-grazing mass) x break size (ha) (4.2)

### 4.4.3 Plant sub-sampling and analyses

Ryegrass was sampled for dry matter and nutritive value by random grab samples collected by hand at grazing height which were bulked and separated into three sections to determine DM % (oven-dried at 60 ℃ for 48 hours), chemical composition and botanical composition. Botanical components were sorted (perennial ryegrass, white clover, were oven-dried to calculate relative abundance in the sward. The third sample was frozen and stored until freeze-dried, ground and analysed by near-infrared spectroscopy (NIRS. Model: FOSS NIRS Systems 5000, Maryland USA).

Daily refusals of FB were collected and weighed to estimate daily FB intake. Three bulbs of FB were randomly selected from the face of the stack to analyse DM, chemical composition and fatty acid content, every third day. Fodder beet bulbs were quartered longitudinally, and two quarters were selected randomly and minced separately using an electric hand blender. One sample was weighed and oven-dried (100℃) over 72 h, to determine DM%, and the second sample was frozen and stored freeze-dried, ground by a centrifugal mill (ZM200 Retsch GmbH; Haan, Germany) to pass through a 1 mm sieve, and then analysed for chemical components (acid detergent fibre: ADF, neutral detergent fibre: NDF, organic matter: OM) using NIRS. The method outlined in AOAC (2003) was used to analyse the crude fat content of FB and herbage. Calibration equations for predicting WSC, CP, ADF, NDF and OM of FB were developed previously on FB bulb samples. The R-squared

values for CP, OM, WSC, NDF and ADF of FB and ryegrass herbage were all above 0.9, and all samples were within the calibration range. The metabolisable energy content of both ryegrass and FB were calculated using the modified ADF (MADF) method; ME (MJ/kg DM) 14.55-0.015*MADF (CSIRO, 2007).

### 4.4.4 Animal Samples and Analyses

Liveweight, and milk yield (kg) were measured automatically at each milking (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden), between days 0-19. A representative sample of milk from each animal was obtained from afternoon and morning milkings every three days using in-line milk meters. Milk samples were sub-sampled, one sub-sample was composited by milking time and treatment and stored at -20℃ until used to analyse milk fatty acid profile. Two sub-samples were used to determine milk urea N (MUN) and the proportion of protein, fat, lactose and milk solids (protein + fat, MS) using Milkoscan (Foss Electric, Hillerod, Denmark, courtesy of Livestock Improvement Corporation, Christchurch, New Zealand). Fatty acid methyl esters of milk and plant material composited by plant type (FB or herbage), diet and period, were prepared by trans-methylation and analysed by gas chromatography (AOAC method 2012.13) (Shimadzu GC-2010, Japan with AOC-20i auto-sampler) using a Varian CP742 silica capillary column (0.25 x 100m x 0.2 µm) courtesy of Fonterra Co-operative Group Ltd. A skimmed sample of milk was frozen at -20℃ until analysed for milk urea nitrogen (MUN) by the enzymatic kinetic method using Randox RX Daytona (clinical assay kit: UR 3825, Randox Rx Daytona, Randox Laboratories, Ltd, United Kingdom). Daily FCE (kg whole milk/kg DMI) was calculated for milk yield by dividing by estimated DMI.

Blood samples were collected in $K_3$EDTA and Li heparin-coated vacuettes via coccygeal vein or artery at approximately 16:00 h on days 2, 11 and 20 of each period, to measure concentrations of non-esterified fatty acid (NEFA) and free amino acids. Collected samples were immediately placed on ice until centrifuged at 3000 × g for 15 minutes at 4ºC. Plasma was transferred to 2 ml Eppendorf tubes and stored at -20ºC until analysed. The concentration of NEFA in plasma was determined following kit instructions (Clinical chemistry assay kit: FA115). The concentration of free amino acids in plasma were determined by liquid

chromatography (HPLC) using a 150 x 4.6mm, C18 3u ACE-111-156 column (Winlab, Scotland), following the method of (Heems et al., 1998).

Ruminal pH was measured every 10 minutes using a wireless bolus (SmaXtec animal care GmbH, Austria). Boli was inserted into the rumen seven days before the experiment and calibrated using a commercial buffer (pH 7.0), following manufacturer instructions. On three occasions of each period, random hand grab samples of rumen digesta were collected from the ventral sac of the rumen at 0400, 0800, 1200, 1600, 2000 and 2400 h each day. Digesta was filtered through Chux cloth (Clorox, Australia) into two 2 mL microtubules to measure $NH_3$ (acidified with 6 M sulphuric acid) and VFA concentration and were stored at -20℃ until analysed. The concentration of VFA was determined by gas chromatography using an SGE BP21 30 m x 530 µm x 1.0 µm wide-bore capillary column using an autosampler (AOC-20i) fitted to a Shimadzu GC-2010 gas chromatograph (Kyoto, Japan). Briefly, samples were thawed overnight at -4℃ and centrifuged at 13,000 rpm for 30 minutes at 4℃ (Beckman centrifuge JA20 rotor). 100 µl of supernatant was collected in a 2 ml Eppendorf tube, 20µl of internal standard (2-methylvaleric acid) and 40 µl of metaphosphoric acid were added, and then the solution was vortexed for 30 minutes. Samples were diluted with acetone and water then vortexed again and passed through a 0.2 µm nylon syringe filter. Ammonia (Enzymatic UV method) and L-lactate (Enzymatic determination method) concentrations of rumen fluid were determined enzymatically using Randox Daytona analyses, following kit instructions ($NH_3$ clinical assay kit: AM 3979, L-lactate clinical assay kit: LC 3985).

### 4.4.5 Statistical Analysis

Response variables were analysed in R (r Core Team, 2018, v. 3.4.4.). Variables were analysed as a factorial arrangement between diet and stage of adaptation. Apparent DMI, herbage intake (HI), FCE and NEFA, AA, milk FA and milk constituents of composited milk samples were analysed by mixed-effects ANOVA using the 'lme' function of the lme4 package (Bates et al., 2015). Treatment (i.e. diet), adaptation stage, the diet × adaptation stage interaction, and period were fixed effects, and the individual cow was a random effect. Variables analysed at the individual cow level such as; botanical components, fermentation end-products (VFA and $NH_3$), ruminal pH (mean, min, max and duration of pH <6.0, <5.8 and <5.6), milk yield and composition (fat, protein, MS and lactose), were analysed using the generalised linear mixed

modelling function of the 'lme4' package. Diet, adaptation stage, the diet × adaptation stage interaction, and period were fixed effects and cow was treated as a random effect. For repeated measurements within a day (VFA, $NH_3$, and ruminal pH), time was also included as a fixed effect and the interaction between time, diet and adaptation stage were also assessed. Several rumen lactate samples were below the detectable limit, and a zero-inflated generalised linear mixed model in the 'glmmTMB' package was instead used to prevent over-dispersion (Brooks et al., 2017). Least square means were generated using the 'emmeans' package of R (Lenth, 2018) and upon the significance of the ANOVA, means were separated using pairwise contrasts. Differences were declared significant if $P < 0.05$ and tendencies were $0.05 < P < 0.1$.

## 4.5 Results

One cow in the first period developed SARA, pH <5.5 for 240 minutes per day on day 10 of adaption, her FB allocation was reduced to 3 kg DM which was maintained until the end of the experiment. Another cow from the second period also developed SARA towards the end of adaptation (days 14-19) pH was <5.5 for between 110 to 190 minutes per day, her allocation of FB was also reduced. Neither cow was removed from the experiment because pH was stabilised without intervention, which is a definitive SARA characteristic.

### 4.5.1 Feed measurements

While pre-grazing herbage mass was similar between treatments, pasture utilisation delined and grazing residuals increased with the FBH treatment ($P < 0.001$, Table 4.1). Ryegrass accounted for > 90% of the biomass in the first period and 85% of the biomass in the second period (Table 4.1). The DM content of FB bulb increased 38.6% from period 1 to period 2, while DM content of herbage was similar for both diets. Proportions of ADF and NDF of the sward and FB bulb increased, while the ME content of herbage declined between periods. Based on a random sampling of the grazed sward horizon, the herbage energy content was >11.0 MJ ME/kg DM, and apparent ME content of FB bulb exceeded 13.0 MJ ME/kg DM. The experimental period did not alter the OM, WSC, CP or N content of herbage or FB. Herbage fatty acid content of FBH and HO diets, including FB bulb, are presented in Table 4.1. The content of FA in herbage was 83% greater than FB bulb. The herbage grazed by the FBH

treatment contained greater amounts of free fatty acids (14.2%) compared with herbage grazed by HO cows (Table 4.1).

### 4.5.2 Estimated intake and milk production

There was no effect of diet on mean daily liveweight or mean seven-day liveweight. However, daily liveweight increased between stages one and two but remained consistent between stages two and three of adaptation (Table 4.2). The significant interaction of liveweight and period reflects a 2.2% decline from period 1 to period 2. Herbage intake was not restricted in either period as evidenced by low herbage utilization and high post-grazing residuals, which exceeded target levels of 1550 kg DM/ha by between 30 - 100%. Intake of FB bulb accounted for 22.2 (stage 1), 32.0 (stage 2) and 35.8% (stage 3) of daily DMI (Table 4.2). The utilisation of FB was high during Stage 1 of adaptation when the allocation was less than 25% of the diet, but after allocation reached 5.5 kg DM/cow/d, utilization became more variable (Table 4.2). Across each stage of adaptation, cows refused 3.63%, (stage 1), 14.98% (stage 2) and 13.1 % (stage 3) of FB bulb offered (Table 4.2). Adaptation to FB bulb caused substitution of herbage for FB bulb by 0.57 $\pm$ 0.1 during stage one, 0.47 $\pm$ 0.14 in stage two and 0.31 $\pm$ 0.22 kg DM herbage/kg DM FB in stage three (mean $\pm$ SE). The effect of adaptation stage or period was not significant, and a wide range of SE between days was observed (-2.70 to +8.57 kg DM/kg DM FB).

Table 4.1. Pre and post-grazing pasture mass, botanical composition (%) of sward and chemical composition[1] (%) including free fatty acids[2] (mg/g DM) of fodder beet root (FB) and herbage that was fed as a herbage only diet (HO) or herbage fed to cows supplemented with FB (FBH). *P* <0.05 *; *P*< 0.01**; *P* <0.001 ***

| | **Period 1** | | | | **Period 2** | | | | ***P*-Value** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Herbage | | FB bulb | | Herbage | | FB Bulb | | | |
| **Diets** | HO | FBH | FBH | **SE[3]** | HO | FBH | FBH | **SE** | Diet | Period |
| Pre-grazing (kg DM/ha) | 5497 | 5453 | - | 86 | 3478 | 3596 | - | 61 | 0.14 | *** |
| Post-grazing (kg DM/ha) | 2823 | 3050 | - | 68 | 1953 | 2277 | - | 59 | *** | *** |
| Area (m2/cow/d) | 53.6 | 52.2 | - | 1.06 | 76.3 | 73.4 | - | 1.71 | 0.18 | *** |
| Allocation (kg DM/cow) | 26.4 | 26.0 | - | 0.38 | 29.1 | 28.7 | - | 0.45 | 0.52 | *** |
| **Sward Botanical** | | | | | | | | | | |
| Ryegrass % DM | 91.6 | 95.2 | - | 2.04 | 86.4 | 85.6 | - | 1.96 | 0.31 | 0.26 |
| White clover % DM | 1.36 | 1.14 | - | 1.67 | 5.02 | 6.14 | - | 2.08 | 0.71 | 0.13 |
| Weeds % DM | 3.96 | 3.73 | - | 2.83 | 8.74 | 10.6 | - | 3.39 | 0.62 | 0.06 |
| Dead % DM | $5.27^{a}$ | 2.89 | - | 0.707 | $1.52^{b}$ | 1.95 | - | 0.914 | 0.38 | 0.14 |
| **Chemical composition** | | | | | | | | | | |
| DM % DM | $14.7^{c}$ | $14.2^{c}$ | $12.7^{a}$ | 0.36 | $17.5^{d}$ | $18.1^{d}$ | $20.7^{b}$ | 0.60 | 0.39 | *** |
| OM % DM | $91.5^{b}$ | $91.4^{b}$ | $94.2^{a}$ | 0.26 | $91.8^{b}$ | $91.7^{b}$ | $93.7^{a}$ | 0.29 | 0.66 | 0.75 |
| ADF % DM | $21.0^{c}$ | $21.2^{c}$ | $7.81^{a}$ | 0.123 | $23.3^{d}$ | $23.6^{d}$ | $8.15^{b}$ | 0.130 | 0.12 | *** |
| NDF % DM | $36.6^{d}$ | $37.7^{c}$ | $13.0^{a}$ | 0.185 | $41.7^{e}$ | $41.8^{e}$ | $14.0^{b}$ | 0.241 | 0.07 | *** |
| WSC % DM | $21.1^{b}$ | $20.6^{b}$ | $63.9^{a}$ | 0.39 | $20.5^{b}$ | $20.2^{b}$ | $59.4^{a}$ | 0.41 | 0.36 | 0.15 |
| CP % DM | $15.6^{b}$ | $16.0^{b}$ | $8.23^{a}$ | 0.27 | $15.7^{b}$ | $15.5^{b}$ | $9.39^{a}$ | 0.37 | 0.69 | 0.29 |
| ME (MJ/kg DM) | $11.2^{a}$ | $11.2^{a}$ | $13.4^{b}$ | 0.03 | $11.1^{a}$ | $11.0^{a}$ | $13.2^{b}$ | 0.03 | *** | *** |
| Fat % DM | $2.30^{c}$ | $2.72^{b}$ | $0.59^{a}$ | 0.084 | $2.12^{c}$ | $2.54^{b}$ | $0.40^{a}$ | 0.088 | *** | 0.13 |
| ΣSFA (mg/g DM) | $4.32^{b}$ | $4.49^{b}$ | $0.66^{a}$ | 0.077 | $4.27^{a}$ | $4.60^{a}$ | $0.76^{b}$ | 0.077 | *** | 0.07 |
| ΣMUFA (mg/g DM) | $1.04^{d}$ | $1.17^{f}$ | $0.43^{b}$ | 0.024 | $0.95b^{c}$ | $1.09^{e}$ | $0.34^{a}$ | 0.0243 | *** | *** |
| ΣPUFA (mg/g DM) | $9.97^{e}$ | $13.21^{f}$ | $1.28^{b}$ | 0.100 | $9.07^{c}$ | $9.76^{d}$ | $0.85^{a}$ | 0.100 | *** | *** |
| ΣTotal FA (mg/g DM) | $18.9^{d}$ | $21.6^{f}$ | $3.29^{b}$ | 0.47 | $16.8^{c}$ | $19.5^{e}$ | $1.19^{a}$ | 0.47 | *** | *** |

[a-e] Means within rows with different superscripts are significantly different (P<0.05). [1] ADF: Acid detergent fibre, NDF: neutral detergent fibre, WSC: water-soluble carbohydrate, OM: organic matter, CP: crude protein, N: nitrogen, ME: metabolisable energy. [2] ΣSFA: the sum of saturated fatty acids; ΣMUFA: Sum of mono-unsaturated fatty acids; ΣPUFA: Sum of poly-unsaturated fatty acids; ΣTotal FA: Sum of all fatty acids. [3] SE: Standard error of the mean.

Table 4.2. Animal liveweight (kg) daily yield of milk and milk solids (MS), estimated daily allocation (HA; kg DM/cow) and apparent intake of herbage (HI), fodder beet (FB) and total dry matter (DMI) during adaptation to either grazed herbage + FB (FBH) or herbage only diet (HO).

| **Adaptation stage[1]** | **Stage one** | | | **Stage two** | | | **Stage three** | | | ***P*-Value** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Diet** | HO | FBH | **SE[2]** | HO | FBH | **SE** | HO | FBH | **SE** | Diet | Period | Stage | D x S[3] |
| Liveweight (kg) | 478[a] | 480[a] | 4.2 | 487[a] | 488[a] | 5.3 | 486[a] | 484[a] | 6.4 | 0.45 | *** | ** | 0.87 |
| 7d LWT[4] (kg) | 493[a] | 494[a] | 3.1 | 496[a] | 498[a] | 3.3 | 497[a] | 499[a] | 3.5 | 0.40 | *** | ** | 0.78 |
| DMI[5] (kg DM/d) | 13.8[a] | 14.5[b] | 0.31 | 11.6[c] | 15.3[b] | 0.42 | 11.6[c] | 15.8[d] | 0.61 | *** | *** | 0.55 | 0.07 |
| HI (kg DM/d) | 13.9[b] | 11.3[a] | 0.39 | 11.6[c] | 10.5[c] | 0.51 | 11.6[c] | 10.1[c] | 0.747 | *** | ** | * | 0.64 |
| FB (kg DM/d) | 0[b] | 3.2[a] | 0.12 | 0[b] | 4.9[c] | 0.21 | 0[b] | 5.7[d] | 0.31 | | ** | *** | |
| FB utilisation % | | 94.4 | 2.22 | | 80.0 | 2.12 | | 82.7 | 1.72 | | 0.52 | *** | |
| HA (kg DM/d) | 18.5[a] | 17.8[a] | 0.28 | 16.6[b] | 16.4[b] | 0.40 | 15.6[b] | 16.5[b] | 0.61 | 0.27 | 0.38 | *** | 0.28 |
| Fat (%) | 5.09 | 4.57 | 0.155 | 4.47 | 4.55 | 0.220 | 4.46 | 4.59 | 0.311 | 0.13 | 0.19 | 0.57 | 0.20 |
| Fat (kg) | 1.29[a] | 1.21[a] | 0.034 | 1.06[b] | 1.06[b] | 0.049 | 1.10[b] | 1.08[b] | 0.067 | 0.22 | *** | 0.11 | 0.51 |
| Protein (%) | 3.68 | 3.76 | 0.053 | 3.79 | 3.67 | 0.075 | 3.55 | 3.71 | 0.106 | 0.08 | 0.50 | 0.52 | 0.92 |
| Protein (kg) | 0.94[a] | 1.00[a] | 0.026 | 0.88[b] | 0.88[b] | 0.036 | 0.86[b] | 0.89[b] | 0.052 | 0.16 | * | * | 0.68 |
| Lactose (%) | 5.12[a] | 5.05[b] | 0.023 | 5.14[a] | 5.08[b] | 0.033 | 5.08[b] | 5.04[b] | 0.047 | * | 0.48 | ** | 0.88 |
| Lactose (kg) | 1.30[a] | 1.34[a] | 0.029 | 1.23[b] | 1.18[b] | 0.041 | 1.23[b] | 1.21[b] | 0.058 | 0.89 | ** | ** | 0.53 |
| MS (%) | 8.87 | 8.43 | 0.158 | 8.26 | 8.42 | 0.224 | 8.09 | 8.38 | 0.316 | 0.33 | 0.15 | 0.52 | 0.18 |
| MS (kg) | 2.23[a] | 2.20[a] | 0.046 | 1.94[b] | 1.94[b] | 0.066 | 1.94[a] | 1.99[a] | 0.093 | 0.88 | *** | * | 0.87 |
| Milk (kg) | 25.4[a] | 26.5[a] | 0.56 | 23.9[b] | 23.3[b] | 0.80 | 24.2[a] | 24.0[a] | 1.13 | 0.51 | ** | ** | 0.46 |
| MUN | 5.3[a] | 7.3[a] | 1.09 | 8.2[ab] | 4.2[a] | 1.52 | 4.6[a] | 4.0[a] | 2.15 | 0.93 | 0.49 | 0.90 | 0.09 |
| FCE (kg milk/kg DMI) | 2.00[b] | 1.78[ab] | 0.149 | 2.07[b] | 1.55[a] | 0.154 | 2.10[b] | 1.61[a] | 0.190 | *** | 0.60 | *** | * |

[a-e] means in the same row with different superscripts differ (P < 0.05). P < 0.05 *; P < 0.01**; P < 0.001 ***.
[1] Stage one: days 1-12; stage two: days 13-17; stage three: days 18-20. [2]SE: Standard error of estimated marginal means. [3]Diet by adaptation stage interaction
[4]Average liveweight over seven days. 4DMI was estimated based on Estimated DMI = (Pre-grazing – Post-grazing mass) x break size (ha).

Apparent DMI was not affected by diet during stage one, but FBH increased DMI by 23.5% during stage two and by 25% during stage three of adaptation compared with HO (Table 4.2). Significant diet by day interactions was still apparent by day 19 of adaptation (Figure 4.1). Apparent herbage intake of cows fed HO were greater during stage one, two ($P$ = 0.075) and three ($P$ < 0.05) of adaptation to the FBH diet. Despite greater estimated DMI, milk yield of cows fed FBH were similar to those fed HO, between the second and third stages of adaptation (Table 4.2). Milk response (kg milk/kg DM of FB bulb) was not different between stages of adaptation or period, and averaged 0.46 $\pm$ 0.13 in stage one, 0.27 $\pm$ 0.18 in stage two and 0.39 $\pm$ 0.29 kg milk/kg DM FB during stage three and ranged from -2.13 to 3.76 kg milk/kg DM FB. Significant effects of adaptation stage were detected for milk solids response which declined between stage one (0.13 kg MS/kg) and stage two (0.07 kg MS/kg) but was maintained in stage three of adaptation (0.07 kg MS/kg DM FB; $P$ = 0.03). Milk solids response also declined between period one and period two (0.14 versus 0.03, kg MS/kg DM FB; $P$ <0.001) and ranged between -0.19 to and 0.77 kg MS/kg DM FB. Apparent DMI varied between individuals and stage of adaptation, the coefficient of variation of the FBH treatment was greater than HO at stage 3 of adaptation (21.7 versus 27.9%)

Interaction of diet by stage or diet by day was not significant ($P$ > 0.10) for milk constituents (fat, protein lactose) although, FBH tended ($P$= 0.13) to reduce milk fat % during stage 1 of adaptation (Table 4.2). Milk fat (kg/d), lactose (kg/d), MS (kg/d) and total solids (kg/d) declined ($P$ < 0.05) between stages one and two but increased ($P$ < 0.05) between stages two and three of adaptation (Table 4.2). Supplementation of herbage with FB increased the fat % of bulk milk compared with HO but was not affected by stage of adaptation although, fat yield declined with stage and was reduced by FBH compared with HO (Table 4.2).

Milk composition from individual animals was not affected ($P$ > 0.10) by treatment, other than lactose %, which declined ($P$ < 0.05) with FBH although, the effect of diet on lactose yield was not significant ($P$ > 0.10). Significant ($P$ < 0.05) period effects were detected for fat (kg), protein (kg), kg MS, total solids, the proportion of lactose and yield of whole milk (Table 4.2). Milk solid yield (kg/day) declined ($P$ < 0.05) 11.3% across all animals from day one to day 20 and mean solids yield also declined 5.76% between periods ($P$ < 0.01). However, significant

dietary interactions of MS and percentage of MS were not detected ($P > 0.10$). The FBH diet reduced FCE of milk compared with HO by 23%. There was no interaction between diet or adaptation stage, and the stage of adaptation did not impact FCE, $P > 0.05$ (Table 4.2).

### 4.5.3 Milk fatty acids

There was minimal diet by day or diet by stage interaction for all fatty acids except for the sum of small chain fatty acids which were transiently reduced by the FBH diet during stage two and returned to HO levels by stage three of adaption (Table 4.3). The concentration of conjugated linoleic acid (CLA) declined with the stage of adaptation ($P < 0.05$), but there was no significant effect of diet. Alternatively, the concentration of palmitic acid (C16:0) increased slightly between stage one and three, due to a tendency ($P = 0.06$) for cows fed FBH in stage one to produce greater palmitic acid concentrations HO. Across all adaptation stages, the FBH diet reduced proportions of long-chain and increased the proportions of medium-chain fatty acids compared with HO (Table 4.3). The proportion of saturated fatty acids (SFA) were increased ($P < 0.05$) by FBH, and the SFA content of milk tended ($P = 0.06$) to increase with adaptation stage (Table 4.3). The milk from cows fed FBH contained greater ($P < 0.05$) concentrations of lauric (C12:0), myristic (C14:0) and palmitic acids (C16:0) compared with milk produced from the HO treatment (Table 4.3). The concentration of branched-chain and trans-FA was not ($P > 0.10$) affected by diet or stage of adaptation, although their concentration tended to increase with adaptation stage (Table 4.3). The FBH diet increased the MUFA content of milk compared with HO, but the PUFA content of milk was not ($P > 0.10$) altered by diet (Table 4.3).

### 4.5.4 Ruminal pH and VFA patterns

Diet by adaptation stage interactions ($P < 0.05$) were detected for ruminal pH and the duration of low pH (Table 4.4). Mean ruminal pH increased across all dietary treatments between stages one and three of adaptation ($P < 0.01$). During the first 12 days of adaptation, mean ruminal pH of cows fed FBH was greater ($P < 0.05$) than cows fed HO. However, mean ruminal pH of cows fed FBH declined ($P < 0.05$) compared with HO during stages two and three (Table 4.4). Significant diet effects on zenith pH were not apparent ($P > 0.10$), although the daily zenith of ruminal pH increased ($P < 0.05$) with the stage of adaptation. The FBH diet reduced ($P < 0.05$) daily nadir of pH by during stage two (5.47 versus 5.64) and stage three

Table 4.3. Change of total small (< C10), medium (< C16 long-chained long-chain (> C16) fatty acids of milk during dietary adaptation to either herbage only (HO) or herbage + fodder beet bulb (FBH) diet.

| **Adaptation stage**[1] | **Stage 1** | | | **Stage 2** | | | **Stage 3** | | | ***P*-Value** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HO | FBH | **SE**[2] | HO | FBH | **SE** | HO | FBH | **SE** | Diet | Stage | D x S |
| g/100g of FA | | | | | | | | | | | | |
| Σ Small chain | 7.26$^{a}$ | 7.41$^{a}$ | 0.108 | 7.42$^{a}$ | 6.81$^{b}$ | 0.153 | 7.26$^{a}$ | 7.23$^{a}$ | 0.216 | 0.37 | 0.22 | * |
| Σ Long chain | 32.8$^{b}$ | 28.2$^{a}$ | 1.47 | 32.4$^{b}$ | 28.0$^{a}$ | 2.07 | 26.4$^{a}$ | 23.5$^{a}$ | 2.93 | * | 0.08 | 0.94 |
| Σ Med chain | 52.3$^{a}$ | 55.8$^{ab}$ | 1.24 | 52.6$^{a}$ | 57.4$^{ab}$ | 1.75 | 58.7$^{b}$ | 60.6$^{b}$ | 2.47 | * | * | 0.80 |
| Σ Branched | 2.02$^{a}$ | 1.88$^{a}$ | 0.060 | 2.94$^{a}$ | 1.97$^{a}$ | 0.084 | 1.75$^{ab}$ | 1.71$^{ab}$ | 0.119 | 0.27 | 0.08 | 0.54 |
| Σ Trans | 3.58 | 3.49 | 0.234 | 3.77 | 3.17 | 0.331 | 2.57 | 2.67 | 0.468 | 0.42 | 0.06 | 0.59 |
| Σ Saturated | 69.8$^{b}$ | 72.5$^{a}$ | 1.13 | 69.8$^{b}$ | 73.2$^{a}$ | 1.60 | 75.3$^{a}$ | 75.8$^{a}$ | 2.26 | * | 0.06 | 0.75 |
| Σ MUFA | 22.0$^{b}$ | 19.1$^{a}$ | 1.11 | 21.7$^{b}$ | 18.8$^{a}$ | 1.56 | 17.1$^{ab}$ | 16.0$^{a}$ | 2.21 | * | 0.09 | 0.87 |
| Σ PUFA | 2.35 | 2.35 | 0.093 | 2.56 | 2.19 | 0.132 | 2.10 | 2.07 | 0.186 | 0.29 | 0.19 | 0.30 |
| g/100g of FA | | | | | | | | | | | | |
| C4 | 3.63$^{a}$ | 3.37$^{b}$ | 0.104 | 3.66$^{a}$ | 3.10$^{b}$ | 0.147 | 3.50$^{a}$ | 3.42$^{ab}$ | 0.208 | ** | 0.61 | 0.33 |
| C6 | 2.26$^{b}$ | 2.43$^{a}$ | 0.038 | 2.33$^{a}$ | 2.28$^{a}$ | 0.054 | 2.33$^{a}$ | 2.35$^{a}$ | 0.076 | * | 0.73 | 0.06 |
| C8 | 1.38$^{b}$ | 1.61$^{a}$ | 0.044 | 1.44$^{a}$ | 1.42$^{a}$ | 0.062 | 1.43$^{a}$ | 1.46$^{a}$ | 0.087 | ** | 0.50 | 0.07 |
| C10 | 3.47$^{b}$ | 4.38$^{a}$ | 0.177 | 3.63$^{a}$ | 3.89$^{a}$ | 0.251 | 3.93$^{a}$ | 4.33$^{a}$ | 0.355 | ** | 0.50 | 0.32 |
| C10:1 | 0.26$^{b}$ | 0.32$^{a}$ | 0.013 | 0.28$^{a}$ | 0.28$^{a}$ | 0.019 | 0.28$^{a}$ | 0.28$^{a}$ | 0.027 | * | 0.79 | 0.15 |
| C12 | 4.32$^{b}$ | 5.49$^{a}$ | 0.285 | 4.25$^{a}$ | 4.92$^{a}$ | 0.404 | 5.01$^{a}$ | 5.67$^{a}$ | 0.571 | ** | 0.32 | 0.72 |
| C14 | 12.3$^{a}$ | 13.5$^{b}$ | 0.36 | 12.4$^{a}$ | 13.7$^{b}$ | 0.51 | 14.0$^{b}$ | 14.4$^{bc}$ | 0.73 | ** | 0.09 | 0.78 |
| C14:1 *cis* 9 | 0.65$^{a}$ | 0.90$^{b}$ | 0.063 | 0.76$^{a}$ | 0.84$^{b}$ | 0.089 | 0.59$^{a}$ | 0.86$^{b}$ | 0.126 | * | 0.73 | 0.37 |
| C15:0 | 1.12$^{a}$ | 1.20$^{a}$ | 0.044 | 1.12$^{a}$ | 1.22$^{a}$ | 0.063 | 1.18$^{a}$ | 1.34$^{a}$ | 0.089 | 0.06 | 0.39 | 0.86 |
| C16:0 | 29.7$^{a}$ | 30.7$^{a}$ | 0.71 | 30.0$^{a}$ | 32.7$^{a}$ | 0.98 | 33.9$^{b}$ | 34.4$^{b}$ | 1.38 | 0.08 | ** | 0.57 |
| C16:1 | 1.11 | 1.12 | 0.066 | 1.10 | 1.11 | 0.093 | 0.99 | 1.13 | 0.132 | 0.69 | 0.81 | 0.76 |
| C17:0 | 0.53 | 0.50 | 0.016 | 0.52 | 0.54 | 0.022 | 0.54 | 0.55 | 0.031 | 0.49 | 0.46 | 0.30 |
| C17:1 | 0.21 | 0.19 | 0.014 | 0.20 | 0.19 | 0.019 | 0.18 | 0.21 | 0.028 | 0.50 | 0.84 | 0.46 |
| C18:0 | 9.90$^{a}$ | 8.34$^{b}$ | 0.424 | 9.62$^{a}$ | 8.56$^{a}$ | 0.599 | 8.57$^{a}$ | 7.00$^{a}$ | 0.847 | ** | 0.14 | 0.89 |
| C18:1 *trans* 9 | 0.14 | 0.13 | 0.004 | 0.13 | 0.12 | 0.006 | 0.12 | 0.11 | 0.008 | 0.06 | 0.07 | 0.90 |
| C18:1 *trans* 11 | 3.44 | 3.36 | 0.232 | 3.64 | 3.05 | 0.328 | 2.45 | 2.55 | 0.464 | 0.43 | 0.07 | 0.60 |
| C18:1 *cis* 9 | 16.3$^{b}$ | 13.5$^{a}$ | 1.03 | 15.8$^{a}$ | 13.5$^{a}$ | 1.46 | 12.5$^{a}$ | 11.2$^{a}$ | 2.07 | * | 0.20 | 0.90 |
| C18:1 *cis* 11 | 0.60$^{b}$ | 0.52$^{a}$ | 0.025 | 0.57$^{a}$ | 0.52$^{a}$ | 0.025 | 0.55$^{a}$ | 0.51$^{a}$ | 0.050 | * | 0.80 | 0.79 |
| C18:2 *cis* 9, 12 | 0.49 | 0.46 | 0.018 | 0.51 | 0.46 | 0.026 | 0.53 | 0.54 | 0.036 | 0.09 | 0.17 | 0.63 |
| C20:0 | 0.08 | 0.05 | 0.014 | 0.06 | 0.06 | 0.020 | 0.05 | 0.03 | 0.028 | 0.21 | 0.64 | 0.66 |
| C18:3 *cis* 9, 12, 15 | 0.69 | 0.66 | 0.033 | 0.76 | 0.67 | 0.047 | 0.79 | 0.69 | 0.067 | 0.17 | 0.39 | 0.66 |
| CLA[3] | 1.18$^{a}$ | 1.23$^{a}$ | 0.080 | 1.29$^{a}$ | 1.06$^{a}$ | 0.113 | 0.80$^{b}$ | 0.85$^{b}$ | 0.160 | 0.79 | * | 0.35 |

$^{a-b}$ means in the same row with different subscripts differ ($P$ <0.05). $P$ <0.05 *; $P$< 0.01**; $P$ <0.001 ***.

[1] Stage one: transition to FB allocation days 1-12; stage two: days 13-17; stage three: days 18-20. [2] SE: Standard error of estimated marginal means. [3] Conjugated linoleic acid *cis* 9 *trans* 11

(5.48 versus 4.71), compared with HO. The gradual increase of FB allocation during stage one caused the duration that pH was <6.0 and <5.8 to increase ($P$ < 0.05) 41 and 16 min/d, respectively, compared with cows fed HO (Table 4.4). However, the duration that pH was <5.6 was also 8.7 minutes longer ($P$ < 0.01) for cows fed FBH than those fed HO, during stage one of adaptation (Table 4.4). The FBH diet increased ($P$ < 0.05) the duration that pH was <6.0 by 49 min/d, the duration that pH was <5.8 by 16 min/d and duration that pH was <5.6 by 19.1 min/d, compared with cows fed HO during stage two of adaptation (Table 4.4). During the third stage of adaptation, the duration that pH was less than 6.0 (20 min/d), 5.8 (9 min/d) and 5.6 (9 min/d) was greater ($P$ < 0.05) from cows fed FBH than those fed HO (Table 4.4). The duration of low pH during stage three declined ($P$ < 0.05) with both diets compared with stage two of adaption. Analysis of diurnal ruminal pH indicated significant ($P$ < 0.05) diet by stage by hour interactions of ruminal pH (Figure 4.2). During the first stage of adaptation, ruminal pH of both diets declined following allocation of herbage or FB, after the morning milking. During stage two, pH patterns were divergent by diet as following the FB meal, pH declined to a nadir of 5.5 and remained below 5.6 until 1400 h while the daily nadir of HO animals averaged 5.7 and pH from both groups remained below 5.8 until after midnight. During the third stage of adaptation, nadir ruminal pH in cows fed FBH reached 5.5 at 1000 h (two hours post-FB allocation), pH was > 5.6 by 0100 h while a nadir pH of 5.7 was maintained for the HO diet (Figure 4.2).

Significant ($P$ < 0.05) diet by adaptation stage interactions and diet by time by stage interactions were detected for all rumen fermentation products except the A: P ratio (Table 4.5). From all samples measured for lactate, 45% were above the detectable limit (> 0.00001 mmol/L) and generally were detected between 1200 h and 1600 h. The FBH treatment increased the concentration of lactate ($P$ < 0.05) on day 11, and lactate concentrations declined again by day 20 of adaptation compared with the HO treatment (Table 4.5). Day of adaptation to the FBH diet had a significant effect ($P$ < 0.05) on all VFA and differences between the treatments were observed from the 11th day of adaptation to FB (Table 4.5). The A: P ratio tended ($P$ = 0.06) to increase with the FBH diet, although differences were small (3.66 versus 3.65). Fodder beet reduced the concentration of propionate by 16% (stage 2) and 12% (stage 3) compared with the HO treatment. The concentration of acetate also declined by 21% (stage 2) and 20% (stage 3) when FBH was fed, compared with HO counterparts. The

FBH diet increased the concentration of butyrate by 16% during stage 2 and 22% during stage 3 (Table 4.5) however, total VFA concentrations declined 13% (stage 2) and 10% compared with cows fed HO (Table 4.5).

Following FB bulb consumption in the morning, concentrations of butyrate and propionate increased, spiking around midday. The concentration of acetate from cows fed FBH, remained constant between 50-60 mmol/L on day 11 of adaptation, while concentrations ranged 80 and 60 mmol/L for cows fed HO. On day 20, concentrations of acetate from cows fed FBH peaked at 70 mmol/L at 1500 h, plateaued during the early evening and declined just before midnight. In comparison, the concentration of acetate from cows fed HO spiked at 90 mmol/L at midday and declined gradually over the following 12 hours (Figure 4.3). Total VFA and ammonia concentrations peaked across both dietary treatments between 0800 h and 1200 h and declined slightly thereafter. The FBH diet increased concentrations of iso-butyrate by 69.2% at midday compared with HO concentrations on day 20 of adaptation (Figure 4.4). The concentration of valerate and hexanoate were increased ($P < 0.05$) by FBH compared with HO, while the concentrations of iso-valerate declined ($P < 0.05$) with FBH (Table 4.5). Dietary differences between valerate, iso-valerate and hexanoate also increased ($P < 0.05$) with the stage of adaptation (Table 4.5; Figure 4.5). Differences of lactate, propionate, total VFA, and hexanoate concentrations between periods were significant ($P < 0.01$).

### 4.5.5 Plasma metabolites

The concentrations of blood metabolites were largely unaffected by diet, and the interaction of diet by adaptation stage was only significant for glycine and serine concentrations (Table 4.6). Glycine concentration of cows fed FBH was 130% greater ($P < 0.05$) than those fed HO at day 11 of adaptation and 28% greater ($P < 0.05$) at day 20. Serine concentrations declined ($P < 0.05$) in cows fed FBH during day 20 (15.2%) compared with cows fed HO. Significant diet effects were also detected for taurine, and isoleucine (Table 4.6), which both increased ($P < 0.05$) with the FBH diet. The effect of adaptation stage was also significant ($P < 0.05$) for NEFA, as concentrations declined across both dietary treatments between days two, 11 and 20 of adaptation. Across the adaptation period, cows fed HO on average had slightly elevated NEFA compared with cows fed FBH ($P < 0.05$).

Table 4.4. Daily mean, nadir and zenith ruminal pH and duration (minutes) that pH was below 6.0, 5.8 and 5.6 during three stages of adaptation to either fodder beet bulb and herbage (FBH) or herbage only (HO) diets.

| **Adaptation Stage**[1] | **Stage one** | | | **Stage two** | | | **Stage three** | | | ***P*-Value** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ruminal pH** | HO | FBH | **SE**[2] | HO | FBH | **SE** | HO | FBH | **SE** | Diet | Stage | D x S |
| Nadir pH | 5.62[a] | 5.57[a] | 0.04 | 5.64[a] | 5.47[b] | 0.04 | 5.71[a] | 5.48[b] | 0.05 | *** | 0.41 | * |
| Zenith pH | 6.35[b] | 6.43[a] | 0.02 | 6.46[c] | 6.48[c] | 0.03 | 6.46[c] | 6.55[d] | 0.04 | 0.15 | *** | ** |
| Mean pH | 6.01[b] | 6.03[b] | 0.002 | 6.09[d] | 6.04[c] | 0.003 | 6.16[f] | 6.08[e] | 0.004 | *** | *** | *** |
| pH <6.0 (min/d) | 516[b] | 475[a] | 2.8 | 392[d] | 441[c] | 3.2 | 349[f] | 369[e] | 3.7 | *** | *** | *** |
| pH<5.8 (min/d) | 142[b] | 126[a] | 1.5 | 118[d] | 134[c] | 1.7 | 99[f] | 108[e] | 1.8 | *** | *** | *** |
| pH <5.6 (min/d) | 12.2[b] | 20.9[a] | 0.7 | 10.9[d] | 30.0[c] | 0.9 | 10.9[d] | 20.8[a] | 0.8 | *** | *** | *** |

[a-f] means in the same row with different subscripts differ ($P$ <0.05). $P$ <0.05 *; $P$< 0.01**; $P$ <0.001 ***.

[1] Stage one: transition to FB allocation days 1-12; stage two: days 13-17; stage three: days 18-20. [2]SE: [2]

[2] Standard error of estimated marginal means

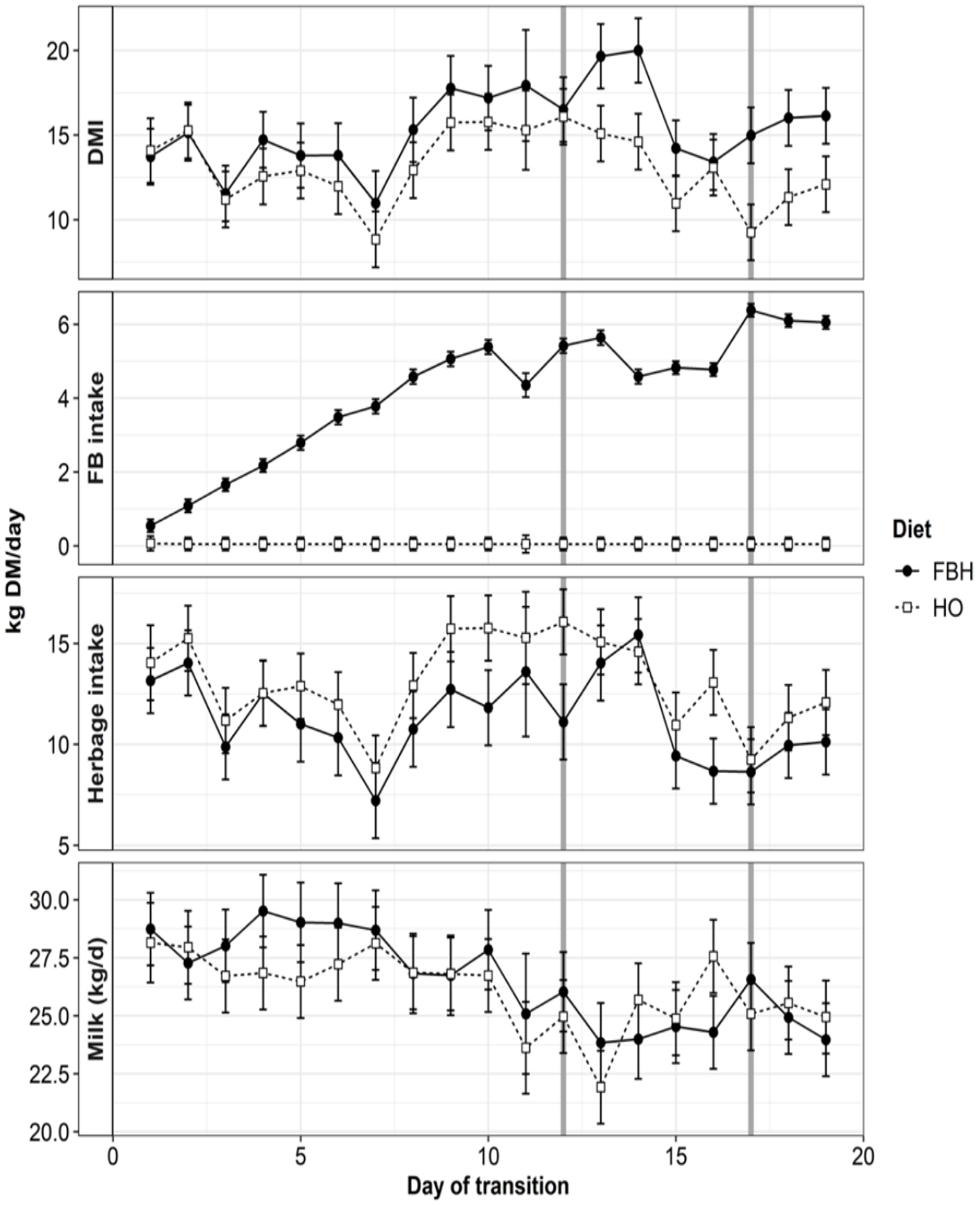


Figure 4.1. Apparent intake of total dry matter (DMI), herbage (HI), fodder beet bulb (FB) and daily milk yield (kg) during dietary adaptation to either herbage only (HO) or herbage and fodder beet diets (FBH). Vertical reference lines represents the stages of FB adaptation where cows reached maximum allocation on day 12 and were fully adapted by day 18.

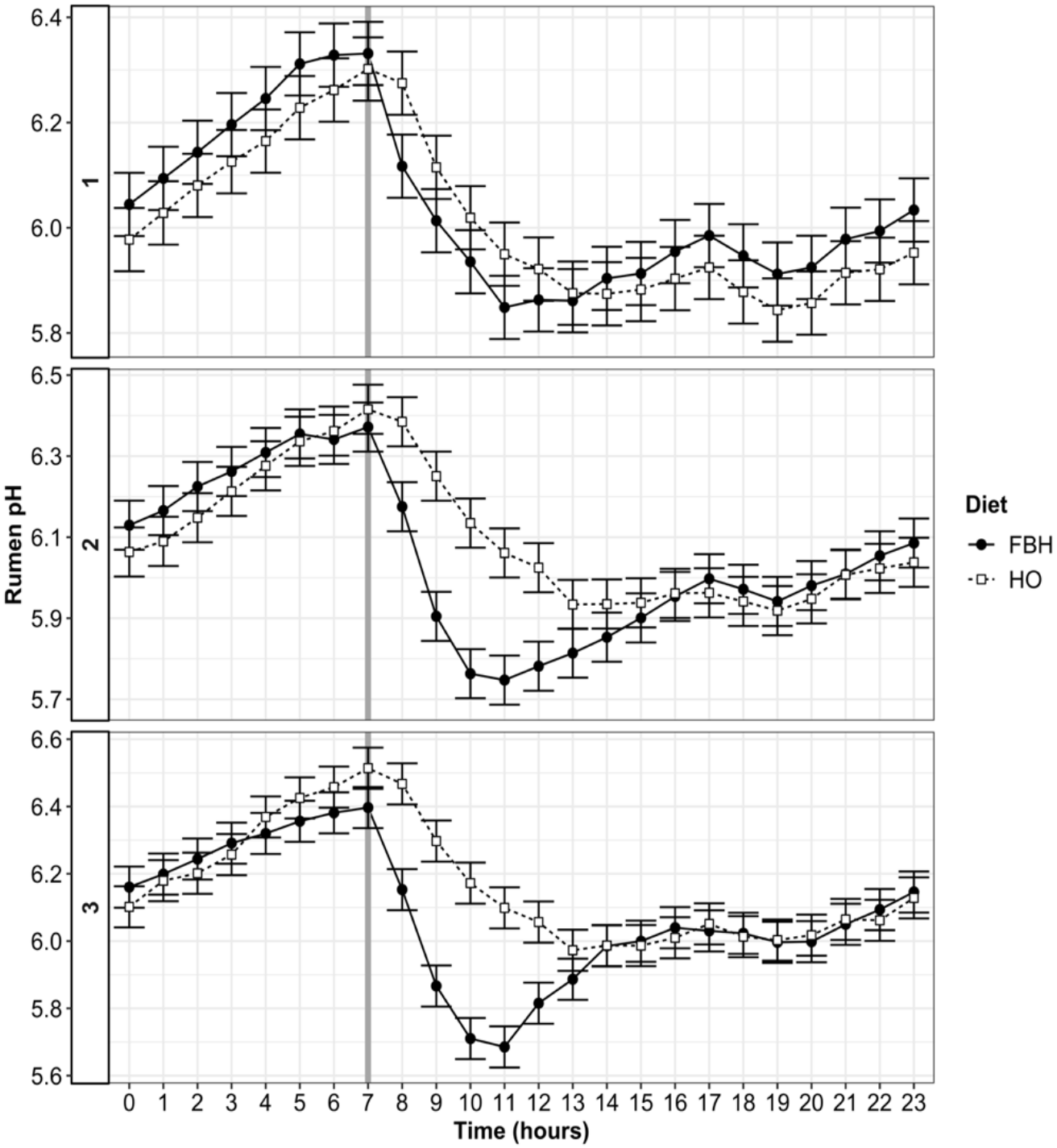


Figure 4.2. Diurnal pattern of ruminal pH during dietary adaptation to herbage and fodder beet bulb (FBH) or herbage only diet (HO). Cows reached maximum FB allocation over 12 days, (Stage 1, top) and acclimatised to their diet during days 13-17 (Stage 2, middle), post-adaptation was assumed during days 18-19 (Stage 3, bottom). Vertical reference line represent the time that FB was fed, or fresh herbage was allocated.

## 4.6 Discussion

This experiment examined the time-dependent changes to rumen function, and fermentation as cows adapted to a diet of herbage and FB bulb using industry recommendations for transitioning (Gibbs, 2014; DairyNZ, 2017). There were no interactions between diet and stage for biohydrogenation intermediates in milk or amino acids (except serine and glycine) in plasma. Such lack of interaction may suggest initial adaptation to the FBH diet although, diet by adaptation stage interactions were still apparent by day 19 and 20 for apparent DMI, FB intake, total VFA and ruminal pH. The occurrence of SARA in two individuals (one during each period) indicates that the null hypothesis should be rejected as the use of industry-recommended transitioning methods did not prevent SARA in individual animals. The following discussion focuses on whether cows fed the FBH diet had fully adapted and some factors which may have prevented individual cows from achieving consistent FB intake and ruminal pH.

### 4.6.1 Ruminal pH and SARA

Consumption of FB bulb during the second and third stage of adaptation caused sub-optimal ruminal pH of all cows fed the FBH diet. Duration of pH <5.6 represent the time frame which fibrolytic activity of the rumen is impaired (Krajcarski-Hunt et al., 2002). Cows fed the FBH diet consistently experienced longer episodes of pH <5.6 compared with animals fed HO (Table 4.4). During day 15 of adaptation, the time that pH was less than 5.6 spiked at 45 minutes/day although intake of FB was consistent at 4.5 kg DM/cow during this time (Figure 4.1). While the FBH diet increased the duration of pH <5.6 compared with HO, the duration that cows experienced low pH was less than that reported by Krajcarski-Hunt et al. (2002) when SARA was deliberately induced (45 versus 594.4 min/day). When pH duration was averaged across all cows, the FBH diet did not cause widespread SARA defined by Gozho et al. (2006) as pH < 5.6 for > 3 hours. However, Zebeli et al. (2012b) found circulating concentrations of pro-inflammatory serum albumin A increased by 0.21 mg/L, each minute that ruminal pH was <6.0. Circulating concentrations of tryptophan and histidine may be linked to concentrations of serum albumin (Aquilani et al., 2019). However, FB did not alter plasma concentrations of either histidine or tryptophan (Table 4.6). The increased duration of low pH caused by the FBH diet may enhance liver-mediated inflammation, laminitis, mastitis, metritis ruminitis and oxidative stress in some individuals within the herd (Gozho et

al., 2005; Plaizier et al., 2008; Khafipour et al., 2009b; Guo et al., 2013) and further evaluation of serum albumin A concentrations are required.

Ruminal pH was reduced following the consumption of FB in the morning; however, ruminal pH of cows fed the HO diet was also low and variable (Table 4.4). Both FBH and HO diets resulted in ruminal pH that was lower than previously reported for housed cows fed increasing proportions of FB and herbage (Pacheco et al., 2020). The continuous measurement of ruminal pH in the current experiment should be more accurate than measurement every 2 hours as practised elsewhere (Duffield et al., 2004; Pacheco et al., 2020). Moreover, Zebeli et al. (2012a) and Mertens (1997) suggest the duration of low ruminal pH also increases when the NDF and physically effective NDF (i.e. stimulates chewing and secretion of saliva) content of herbage is less than 42%. In comparison, the NDF content of herbage fed in the current experiment was between 36-41%, while NDF of FB bulb was comparably less (13-14% NDF) and probably accounted for the generally low pH observed and further decline of the FBH treatment. High nutritive value herbage is readily fermentable and contains minimal amounts of NDF, or ADF and incidence of SARA are probably underestimated in grazing dairy cows (O'Grady et al., 2008). Thus, the low NDF and high digestibility of FB bulb do not appear to complement the low NDF content of herbage, particularly postpartum when the risk of SARA is elevated (Cassida and Stokes, 1996; Penner et al., 2007).

Daily DMI of cows fed FBH was variable (Figure 4.1) and the CV of DMI observed for cows fed FBH was still greater than those fed HO at stage 3 of adaptation (28% and 21%, respectively). While low DMI is generally understood to be the primary symptom of severe SARA, the variation of DMI between days indicates mild SARA. Coombe et al. (2015) and Gozho et al. (2006) reported that DMI increased transiently from 0.8 to 1.0 kg DM, during starch induced SARA. Variation of DMI reflects the cyclical accumulation of VFA within the rumen and their potent anorexigenic control on voluntary intake (Illius and Jessop, 1996; Nocek, 1997; Brown et al., 2000). In the current experiment, daily DMI of cows fed FBH was also cyclic, increasing day 13 of adaptation to 20 kg DM/cow and declining 5 kg DM/cow on day 14. The reduction of DMI from cows fed HO on day 13 may indicate that ruminal sampling on day 11 reduced grazing time. However, disrupted grazing time do not entirely account for the observed variation of intake between treatments or the changes observed between days

13 and 14 (Figure 4.1). However, it should be noted that DMI was also variable across the HO treatment (Figure 4.1) and despite the limited advantage of FB to milk production, FCE may have been confounded in the current experiment due to greater DMI of cows fed FBH.

The high FCE observed in cows fed HO may indicate that cows consumed insufficient DMI to meet energy demands for lactation. The cows in this study were producing 24.5 kg of milk per day which, which based on AFRC formula for a 490 kg cow will have an ME requirement of approximately 200 MJ ME/day (equivalent to 16-17 kg DM/day). The FCE of cows fed HO during stage three was 2.1 (HO) kg milk/kg DM which is greater than the FCE of 1.8 kg milk/kg DMI reported by (Arndt et al., 2015) and FCE of cows fed FBH in the current experiment was less than published results (1.61 kg milk/kg DMI). High FCE was due to similar milk production as cows fed FBH, despite low DMI. While residual post-grazing herbage cover of cows fed HO suggest herbage allocation was not limiting (Table 4.1), low DMI of the HO treatment may have increased mobilisation of adipose tissue and diluted the energy used for maintenance (Bobe et al., 2004). The increased circulation of NEFA observed in cows fed HO may indicate greater energy deficit compared with cows fed FBH. However, increased circulation of NEFA is associated with increased concentration of C18:1 cis 9 in milk (Chilliard et al., 2000; Gross et al., 2011), although this was not observed in cows fed HO (Table 3). Therefore, the increased circulation of NEFA in cows fed HO while statistically significant is probably not biologically meaningful.

### 4.6.2 Patterns of Rumen Fermentation

The 23% reduction of FCE in the FBH treatment may suggest reduced digestion efficiency either due to greater DMI and rumen passage rate (Colucci et al., 1982) or low ruminal pH (Plaizier et al., 2008). While the FBH diet also increased DMI by 25%, this should not 000have caused a near equal decline of FCE. Auldist et al. (2011) supplemented a herbage only diet with low (2.5 kg DM/day) or high (5.0 kg DM/day) amounts of grain during early-lactation (60 DIM) and reported that low and high grain allocation increased DMI by 12% and 20%, respectively, and did not alter FCE compared with cows fed herbage only. The reduced FCE caused by feeding FB is abnormal compared with alternative supplements which may suggest additional factors such as reducing ruminal pH reduced digestion efficiency or an error in the calibration of sward mass and height in the HO treatment. Circulating serine concentrations

also declined 15% in cows fed FBH on day 20 of adaptation. Serine concentrations of all cows were low compared with a previous study of cows fed a high energy total mixed ration (43 versus 75 μmol/L) (Laeger et al., 2012). The reduction of serine in cerebrospinal fluid (Laeger et al., 2012) and plasma (Baird et al., 1972) of lactating dairy cows fed a restricted diet indicate serine is an anorexigenic signal, which may also explain the increased DMI of the FBH treatment. However, further research of the relationships between plasma concentrations of serine, supplementation of FB, the effect of SARA and DMI are needed.

Diurnal patterns of all fermentation end products reflected the time of feeding (FB or herbage), in the morning (Figure 4.3, Figure 4.4). Despite greater apparent DMI, cows fed FBH experienced lower concentrations of total VFA, as both acetate and propionate concentrations declined, compared with cows fed HO. While Pacheco et al. (2020) also reported lower acetate concentration from cows when FB was fed as 23% or 45% of DMI, differences were not as large as observed in the current study.

Table 4.5. Rumen concentration of volatile fatty acids (VFA) and lactate during dietary adaptation to either herbage and fodder beet bulb (FBH) or herbage only diet (HO) diet.

| **Adaptation Stage**[1] | **1** | | | **2** | | | **3** | | | ***P*-Value** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Diet** | HO | FBH | **SE**[2] | HO | FBH | **SE** | HO | FBH | **SE** | Diet | Day | Time | DxDxT[5] |
| Lactate (mol/L) | 0.94[a] | 2.60[a] | 1.851 | 3.20[a] | 84.7[c] | 27.0 | 0.70[a] | 4.35[b] | 2.212 | ** | *** | *** | *** |
| Ammonia (mmol/L) | 4.58[a] | 4.04[a] | 0.368 | 3.42[c] | 2.28[b] | 0.270 | 3.59[c] | 1.53[b] | 0.231 | ** | *** | *** | * |
| tVFA[3] (mmol/L) | 117[a] | 118[a] | 2.5 | 119[a] | 103[b] | 2.6 | 118[a] | 107[b] | 2.6 | *** | * | *** | 0.81 |
| Acetate (mmol/L) | 60.8[a] | 59.[b] | 0.31 | 60.5[a] | 55.0[b] | 0.28 | 63.3[a] | 56.5[b] | 0.29 | *** | *** | *** | *** |
| Propionate (mmol/L) | 21.1[a] | 21.5[a] | 0.22 | 21.2[a] | 20.6[b] | 0.22 | 20.0[b] | 19.8[b] | 0.21 | *** | * | *** | * |
| Iso-butyrate (mmol/L) | 0.73[a] | 0.78[b] | 0.013 | 0.72[a] | 0.50[c] | 0.011 | 0.71[a] | 0.55[c] | 0.011 | *** | *** | *** | *** |
| Butyrate (mmol/L) | 13.3[a] | 14.2[b] | 0.20 | 14.0[b] | 17.6[d] | 0.21 | 12.7[e] | 15.4[c] | 0.19 | *** | *** | *** | ** |
| Iso-valerate (mmol/L) | 1.06[a] | 1.13[ab] | 0.027 | 1.05[a] | 0.68[b] | 0.020 | 1.05[a] | 0.59[b] | 0.019 | *** | *** | *** | *** |
| Valerate (mmol/L) | 1.46[a] | 1.32[ab] | 0.050 | 1.35[a] | 2.38[b] | 0.070 | 1.43[a] | 1.25[c] | 0.073 | *** | *** | *** | 1.00 |
| Hexanoic (mmol/L) | 0.55[a] | 0.60[a] | 0.019 | 0.53[a] | 0.82[b] | 0.023 | 0.50[a] | 0.88[c] | 0.023 | *** | *** | *** | 0.16 |
| A+B/P[4] | 3.54[a] | 3.47[a] | 0.058 | 3.49[a] | 3.62[ab] | 0.062 | 3.81[b] | 3.81[b] | 0.067 | 0.66 | *** | *** | 0.06 |

[a-e] means in the same row with different subscripts differ ($P$ <0.05). $P$ <0.05 *; $P$< 0.01**; $P$ <0.001 ***

[1] Stage one: transition to FB allocation days 1-12; stage two: days 13-17; stage three: days 18-20. [2]SE: Standard error of estimated marginal means. [3] total VFA. [4] Acetate + butyrate: propionate ratio. [5] Diet, day and time interaction

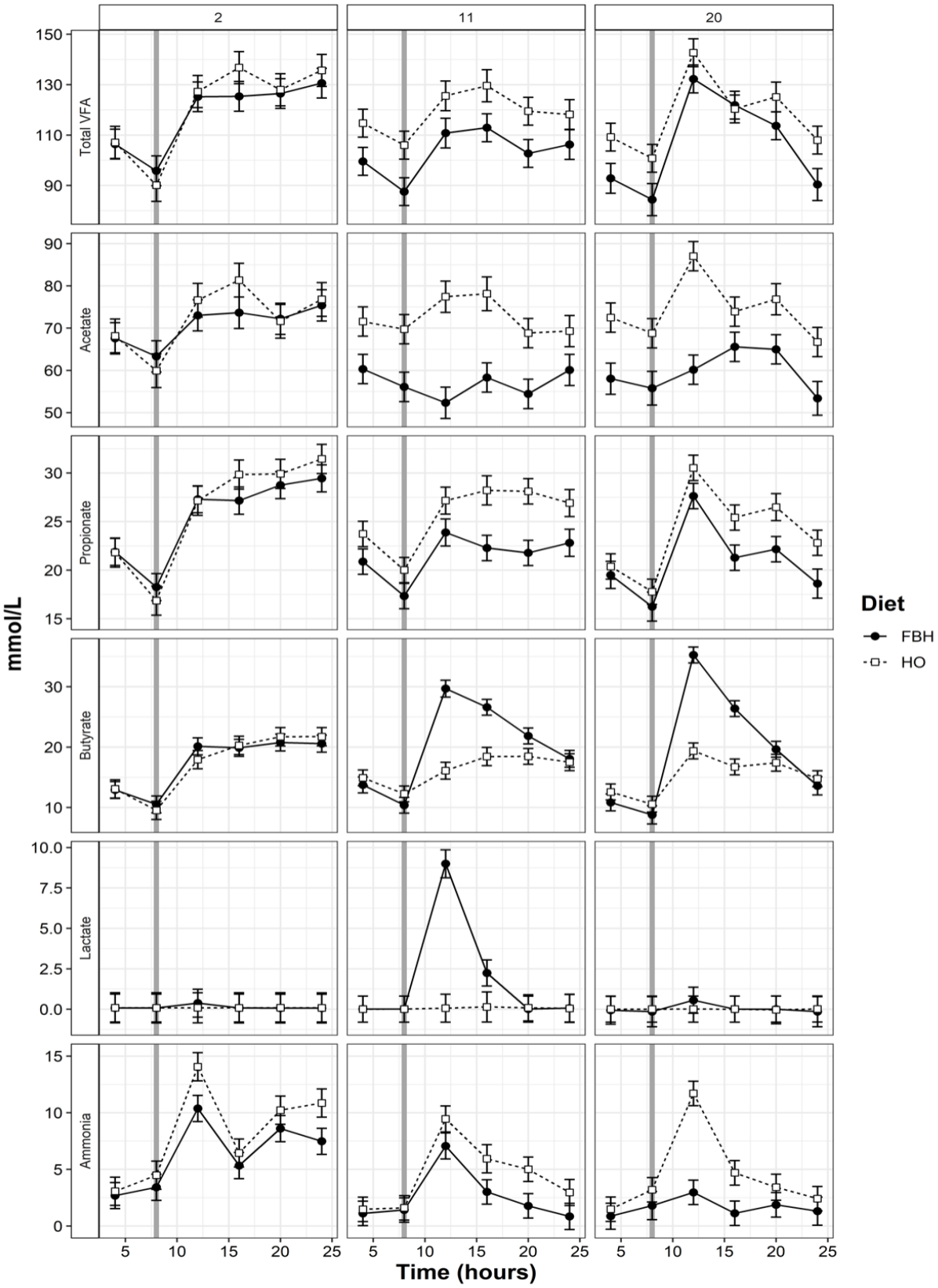


Figure 4.3. Diurnal fluctuation of fermentation-end products and total volatile fatty acid concentrations of rumen fluid collected from cows during adaptation (days 2, 11 and 20) to either herbage and fodder beet bulb (FBH) or herbage only diet (HO). Vertical reference lines represent the time of FB meal, or fresh herbage allocation.

A reduction of total VFA concentration has only been reported for non-lactating dairy cows fed large amounts of FB (>60% DMI) (Pacheco et al., 2020). While the greater concentration of butyrate experienced by cows fed FBH has been previously observed *in vivo* and *in vitro* (Chapter 6), the decline of propionate has not been reported for grazing dairy cows fed FB and herbage (Pacheco et al., 2020). Daily mean butyrate concentrations observed from cows fed FBH were within a similar range (15-17 mmol/L) to the previous study, but butyrate concentration from cows fed HO were slightly greater than those observed by Pacheco et al. (2020) (14.3-15.2 versus 10 mmol/L). While concentrations of propionate did increase following consumption of FB bulbs, cows fed HO experienced a greater spike of propionate following allocation of herbage in the morning, reflecting the high digestibility of spring pasture (Figure 4.3). The concentration of VFA indicates microbial activity but may be confounded by the rate of VFA removal from the rumen, resulting in the underestimation of total VFA concentrations (Dijkstra et al., 1993). Cows fed FBH consumed 12.9% less herbage during stage 3, which does not account for the 26.1% decline in mean acetate concentrations observed on day 20 compared with the HO treatment. The decline of total rumen VFA, FCE and plasma serine concentration and further support the conclusion that FBH impaired rumen function. The spike of lactate concentration coincided with the daily nadir of ruminal pH following FB intake in the morning. A significant, individual cow effect was detected for lactate concentrations; but surprisingly, cows that experienced the greatest lactate concentrations were not always the same animals that developed SARA, indicating individual resilience to low ruminal pH. Unfortunately, D-lactate was not detectable using GC methods, and L-lactate was measured enzymatically instead. While L-lactate found in the rumen is the same form produced from glucose metabolism in muscle, D-lactate is of microbial origin and is not formed by mammalian cells (Slyter and Rumsey, 1991). However, the elevated concentration of L-lactate from animals fed FBH still indicate altered microbial community profiles, although the proportion of D-lactate is expected to increase and L-lactate decline at low ruminal pH (Nocek, 1997). The increase of lactate observed in cows fed FBH was minor compared with previous SARA challenges (Nagaraja and Titgemeyer, 2007) but may have favoured the formation of butyrate, due to increased concentration of $H^+$, osmotic pressure and altered metabolism of the rumen epithelium (Baldwin and Jesse, 1996; Owens et al., 1998; Hegarty and Gerdes, 1999). Lactate was present at minor concentrations in the rumen. Therefore, the low pH of ruminal fluid collected from cows fed FBH probably reflected the accumulation of VFA in the rumen. However, the VFA removal rate may also have increased with the FBH diet,

which would explain why peak VFA concentrations measured at 4 h intervals were still less than the HO treatment.

Butyrate is a favoured energy substrate for epithelial cells and may indicate altered epithelial metabolism (Storm et al., 2011; Schlau et al., 2012; Storm et al., 2012; Penner, 2014). Intra-ruminal administration of butyrate caused a 4-fold proliferation of epithelial cells (Sakata and Tamate, 1998). Specifically, cell thickness of the stratum corneum increased by 15 cells, which is the fourth cornified layer of the rumen epithelia (Gäbel et al., 1987) and is linked to increased localised inflammation, ruminitis and parakeratosis (Ørskov, 1976; Storm et al., 2011). Like propionate, intravenous administration of butyrate causes hyperglycaemia but has also been found to induce a rapid and sustained increase of serum insulin in sheep (Manns, 1964). Insulin sensitivity may also explain the lower circulatory concentration of NEFA observed from cows fed FBH which contrasted to the decline of lactose content that has also been observed previously in cows fed FB (Chapter 3) and has been identified in cows induced with SARA using barley-grain (Guo et al., 2013). Lactose is comprised of a glucose and a galactose residue which are both formed from hepatic oxidation of propionate (Annison and Linzell, 1964) and which declined when FB bulb was fed (Table 4.2). The majority of butyrate formed in ruminal fermentation is used by the rumen epithelium (Baldwin and Jesse, 1996; Owens et al., 1998), and it is not clear whether low ruminal pH and increased concentrations of butyrate may have increased the permeability of the rumen epithelium, altering insulin signalling and epithelial ketogenesis (Baldwin, 1998; Zhang et al., 2019). While the FBH diet appeared to improve postpartum energy status, the reduction of circulating NEFA and the lactose percentage of milk reflect altered fermentation dynamics and reduced substrate availability for hepatic gluconeogenesis.

### 4.6.3 Ruminal adaptation

Of further interest to the time-dependent adaptation of dairy cows fed FB is the absorptive capacity of the rumen wall. Passive diffusion of undissociated VFA (HVFA), and alkalization and buffering of VFA, by phosphate and bicarbonate ($HCO_3^-$) in saliva, are mechanisms which help to stabilise ruminal pH (Owens et al., 1998). However, $HCO_3^-$ mediated transfer of dissociated VFA ($VFA^+$) across the epithelium, removes >50% of all hydrogen ions from the rumen and is a primary mechanism for maintaining ruminal pH (Allen, 1997; Aschenbach et al., 2011; Dijkstra et al., 2012). Both structural (proliferation of epithelial cells, increased papillae size) and width and metabolic adaptations

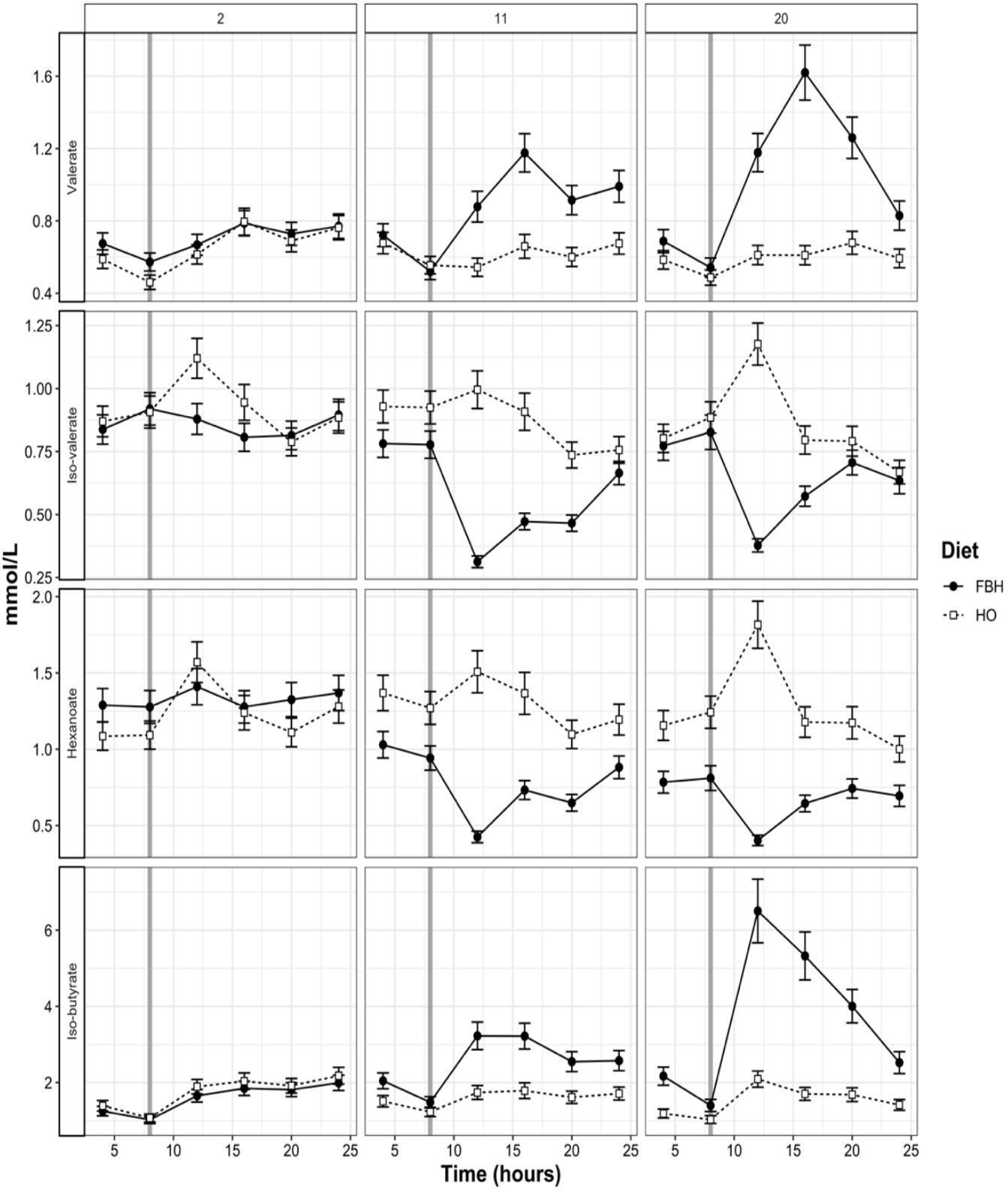


Figure 4.4. Diurnal fluctuation of valerate, iso-valerate, hexanoate and iso-butyrate concentrations of rumen fluid collected from cows during adaptation (days 2, 11 and 20) to either herbage and fodder beet bulb (FBH) or herbage only diet (HO). Vertical reference lines represent the time of FB meal, or fresh herbage allocation.

Table 4.6. Plasma concentration (μmol/L) of amino acids and plasma non-esterified fatty acid (NEFA mmol/L) during dietary adaptation to either a fodder beet bulb and herbage (FBH) or herbage only (HO) diet.

| **Adaptation Stage[1]** | **1** | | | **2** | | | **3** | | | ***P*-Value** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Diet** | HO | FBH | **SE[2]** | HO | FBH | **SE** | HO | FBH | **SE** | Diet | Day | D x D |
| glutamate (μmol/L) | 73.7$^{a}$ | 74.8$^{a}$ | 2.57 | 80.0$^{ab}$ | 87.0$^{b}$ | 2.57 | 78.0$^{b}$ | 85.9$^{b}$ | 2.66 | * | *** | 0.35 |
| Aspartate | 8.11$^{a}$ | 9.72$^{a}$ | 0.765 | 11.26$^{b}$ | 11.33$^{b}$ | 0.765 | 10.69$^{b}$ | 12.28$^{b}$ | 0.793 | 0.10 | ** | 0.52 |
| Cysteine | 99.3 | 99.5 | 3.99 | 106 | 103 | 3.99 | 105 | 101 | 4.13 | 0.47 | 0.42 | 0.85 |
| Asparagine | 8.11$^{a}$ | 9.72$^{a}$ | 0.765 | 11.26$^{b}$ | 11.33$^{b}$ | 0.765 | 10.69$^{b}$ | 12.28$^{b}$ | 0.793 | 0.10 | ** | 0.52 |
| Serine | 43.0$^{a}$ | 47.9$^{a}$ | 3.29 | 44.1$^{a}$ | 43.5$^{a}$ | 3.29 | 42.0$^{a}$ | 35.6$^{b}$ | 3.34 | 0.63 | ** | * |
| Glutamine | 103 | 94.8 | 9.04 | 99.6 | 103 | 9.04 | 93.7 | 86.0 | 9.33 | 0.50 | 0.36 | 0.73 |
| Histidine | 27.0$^{a}$ | 28.0$^{a}$ | 1.30 | 26.6$^{b}$ | 24.1$^{b}$ | 1.30 | 21.3$^{c}$ | 19.3$^{c}$ | 1.34 | 0.24 | *** | 0.28 |
| Glycine | 97.4$^{a}$ | 112$^{a}$ | 13.9 | 79.4$^{a}$ | 182$^{c}$ | 13.9 | 86.6$^{a}$ | 110$^{b}$ | 14.4 | *** | * | ** |
| Threonine | 98.3$^{b}$ | 103$^{b}$ | 8.70 | 103$^{b}$ | 120$^{bc}$. | 8.70 | 91.0$^{a}$ | 89.5$^{a}$ | 8.94 | 0.29 | * | 0.42 |
| Arginine | 49.9 | 53.2 | 4.51 | 54.7 | 54.2 | 4.51 | 54.8 | 48.5 | 4.68 | 0.73 | 0.77 | 0.57 |
| Alanine | 122$^{a}$ | 138$^{a}$ | 12.9 | 162$^{b}$ | 153$^{b}$ | 12.9 | 131$^{a}$ | 114$^{a}$ | 13.4 | 0.60 | ** | 0.23 |
| Taurine | 23.3$^{a}$ | 23.8$^{a}$ | 1.24 | 24.5$^{a}$ | 30.4$^{b}$ | 1.20 | 23.8$^{a}$ | 26.7$^{a}$ | 1.28 | ** | ** | 0.07 |
| Tyrosine | 42.2$^{a}$ | 44.2$^{a}$ | 4.44 | 47.4$^{a}$ | 43.2$^{a}$ | 4.44 | 37.0$^{b}$ | 33.6$^{b}$ | 4.60 | 0.52 | 0.06 | 0.73 |
| Valine | 148 | 167 | 13.8 | 158 | 168 | 13.8 | 147 | 133 | 14.3 | 0.66 | 0.23 | 0.46 |
| Methionine | 18.7$^{a}$ | 19.1$^{a}$ | 1.70 | 20.0$^{a}$ | 21.4$^{a}$ | 1.70 | 15.3$^{b}$ | 14.9$^{b}$ | 1.76 | 0.79 | ** | 0.88 |
| Tryptophan | 42.2$^{a}$ | 44.4$^{a}$ | 4.44 | 47.4$^{a}$ | 43.2$^{a}$ | 4.44 | 37.0$^{b}$ | 33.6$^{b}$ | 5.60 | 0.52 | 0.06 | 0.73 |
| Phenylalanine | 41.3$^{a}$ | 44.3$^{a}$ | 3.83 | 46.5$^{a}$ | 43.8$^{a}$ | 3.83 | 38.2$^{ab}$ | 32.6$^{ab}$ | 3.97 | 0.53 | 0.04 | 0.54 |
| Isoleucine | 93.6 | 103 | 9.76 | 96.1 | 128 | 9.76 | 91.8 | 93.8 | 8.9 | 0.09 | 0.15 | 0.31 |
| Lysine | 31.9 | 41.9 | 6.36 | 35.8 | 39.6 | 6.36 | 24.4 | 20.5 | 6.60 | 0.58 | * | 0.57 |
| Leucine | 93.5 | 104 | 10.2 | 95.4 | 93.2 | 10.2 | 86.8 | 71.4 | 10.6 | 0.74 | 0.15 | 0.47 |
| Proline | 71.0 | 71.8 | 5.11 | 73.4 | 77.2 | 5.11 | 75.3 | 73.9 | 5.25 | 0.75 | 0.61 | 0.82 |
| NEFA (mmol/L) | 0.08$^{a}$ | 0.08$^{a}$ | 0.0064 | 0.08$^{a}$ | 0.05$^{b}$ | 0.0064 | 0.07$^{a}$ | 0.06$^{ab}$ | 0.0066 | * | * | 0.09 |

$^{a-f}$ means in the same row with different subscripts differ (*P* <0.05). *P* <0.05 *; *P*< 0.01**; *P* <0.001 ***. [1] Stage one: transition to FB allocation days 1-12; stage two: days 13-17; stage three: days 18-20. [2]SE: Standard error.

(Dirksen et al., 1985; Dijkstra et al., 1993; Etschmann et al., 2009; Dieho et al., 2016). While morphological changes occur over an extended time frame (16 days – several weeks) postpartum (Dirksen et al., 1985; Dieho et al., 2016), Etschmann et al. (2009) reported 70% of metabolic changes to the rumen epithelium occurred within the first seven days following a change of diet. Therefore, the 12-day transitioning, and 5-day adaption period used in the current experiment should have been sufficient for metabolic development of the rumen epithelium. However, ruminal pH still declined with the FBH diet and transitioning did not prevent SARA in 2 out of 8 cows.

The inability to maintain intake of herbage or FB bulb using individualised feeding methods is concerning for commercial dairy producers who feed FB during lactation, due to the limited clinical symptoms and the apparent individual risk of SARA within the herd. The individualised response to a SARA challenge has been identified in cows fed a starch-rich diet (García et al., 2007; Dohme et al., 2008; Gao and Oba, 2014; Gao and Oba, 2016). Both VFA accumulation and absorption rate are factors associated with the individualised response to a SARA challenge, although the specific mechanisms involved in the response are not yet clear (Gao and Oba, 2016). In commercial dairy systems, group feeding of FB may further enhance the risk of SARA due to both to the increased intake rate caused by competition (Proudfoot et al., 2009) and reduced herbage allocation as FB is often used to mitigate herbage deficits (Chapter 3). Further information regarding individual susceptibility to ruminal acidosis over an extended period is needed to develop FB feeding methods, which prevent SARA in cows predisposed to this disease.

## 4.7 Conclusion

Dairy cows fed moderate allocations of a high sucrose supplement such as FB have increased duration of pH <5.6. The FBH diet reduced FCE, rumen VFA concentrations, and plasma serine concentration, indicating the rumen's impaired fibrolytic activity, even after 20 days following industry recommended methods. Consequently, industry guidelines for feeding FB to early lactation dairy cows can increase animal health risks and reduce animal performance. Further evaluation of industry guidelines for the duration and FB transitioning methods, adaptation and upper limits of daily intake during early lactation are needed.

# Chapter 5

# Rumen function and grazing behaviour of early-lactation dairy cows supplemented with fodder beet bulb

*Accepted for publication in the Journal of Dairy Science (November 2020)*

## 5.1 Abstract

Fodder beet (FB) is a source of readily fermentable carbohydrate that can mitigate early spring herbage deficits and correct the negative energy balance experienced during early-lactation in pastoral dairy systems of New Zealand. However, the low fibre and high soluble carbohydrate content of both FB bulb and spring herbage are factors that promote sub-acute ruminal acidosis (SARA), impairing rumen function and limiting the marginal milk production response to supplement. In a cross-over experiment, eight Holstein Friesian × Jersey early-lactation dairy cows were used to test the effect of supplementing 16 kg DM of a grazed perennial ryegrass-herbage with 6 kg dry matter (DM)/d of FB bulb (FBH) on changes in rumen function and grazing behaviour compared with cows consuming herbage only (HO). Following 20 d of adaptation to diets, DM disappearance (%) of FB bulb (FBH cows only) and herbage were measured *in sacco*, separately. Cows were fasted overnight, and the ruminal contents were bailed the following morning (~09:30 h) again to determine the pool size of volatile fatty acids (VFA), ammonia, and particle size of digesta, as well as to estimate the rate of ruminal outflow and degradation of neutral detergent fibre (NDF). The FBH diet did not alter DMI, milk yield, or milk solid (MS: fat + protein) production compared with HO. Supplementation of herbage with FB reduced ruminal pH compared with HO between ~08:00 h and 13:00 h each day. During each period, one cow experienced severe SARA (pH < 5.6 for >180 min/d) during final adaption to the target FB allocation. The FBH diet reduced the ruminal pool of acetate and ammonia but increased the ruminal pool of butyrate and lactate, compared with HO. When fed FB, cows increased rumination and grazing time while grazing intensity declined, and rumination intensity increased compared with cows fed HO. Despite increased rumination, the comminution of large particles declined 28% between the first and second rumen bailing when cows were fed FB, while *in sacco* DM disappearance of perennial ryegrass

declined 18% compared with cows fed HO. These results indicate that grazing dairy cows supplemented with FB (40% of daily intake) increase rumination and mastication intensity to counteract reduced ruminal degradation of ryegrass herbage due to low ruminal fluid pH.

## 5.2 Introduction

Fodder beet bulb (FB; *Beta vulgaris L.*) is a sugar-dense supplement used to increase the energy intake of lactating dairy cows. In Europe and the United States, FB pulp is fed as a by-product of sucrose, extracted from fresh fodder beets for human consumption or ethanol production. In Europe, older FB varieties such as Mangelwurzels have been grown and fed to livestock since the 18th Century. However, the widespread use of FB to mitigate seasonal herbage deficits in New Zealand has occurred within the last 10 years (Dalley et al., 2019; Waghorn et al., 2019). The popularity of FB in New Zealand is driven by the potential to grow large yields (>20 t DM/ha) of highly utilizable (>90%) bulb with ~ 4 t DM/ha of a leaf which senesces in winter. Fodder beet is sown in spring, and moderate allocations (<40% DMI) are grazed during late lactation. In winter, gradual transitioning feeding regimes are used widely by the dairy industry, enabling non-lactating dairy cows to graze large quantities of FB ( > 70% DMI) that were previously thought to be fatal to livestock (Chakwizira et al., 2013; Gibbs, 2014; Saldias and Gibbs, 2016). The residual FB bulb from winter is also harvested and used to supplement the spring herbage supply to return the paddock to pasture and improve the postpartum negative energy balance of early lactation dairy cows. In New Zealand, harvested FB bulb is generally fed to dairy cows on the paddock using a silage wagon. Feed pads are uncommon in the low-input and minimal infrastructure pastoral dairy systems, which predominate the industry. However, both the crude protein (< 10% DM) and fibre (< 20% DM) content of FB bulb are inadequate for lactating dairy cows. The latter, in conjunction with the high water-soluble carbohydrate content (WSC: > 60 % DM) of FB bulb, are risk factors for ruminal acidosis (Dalley et al., 2019; Waghorn et al., 2019). Ruminal acidosis interferes with healthy rumen function and can impose considerable animal welfare and economic costs to farmers. The physiological mechanisms that regulate ruminal pH and the impact on cow health and rumen function have been explored extensively (Owens et al., 1998; Plaizier et al., 2008; Zebeli and Metzler-Zebeli, 2012b). Briefly, SARA is caused by the rapid accumulation of VFA in the rumen when feeds rich in readily fermentable carbohydrates such as FB, are consumed. The accumulation of VFA causes pH and buffering capacity of the rumen to decline episodically (Owens et al., 1998; Plaizier et al., 2008), but is generally self-corrected. Severe ruminal

acidosis can occur when pH declines below 5.0 as the growth of lactic acid (ten-fold the acidity of other VFA) producing bacteria increase and cause a rapid deterioration of ruminal pH that the cow is unable to correct (Owens et al., 1998). Despite careful transitioning and individualised feeding of FB, acute and SARA have still been reported in both late lactation and non-lactating dairy cows fed either straw and FB or harvested herbage and FB (Waghorn et al., 2018; Waghorn et al., 2018). However, changes to ruminal fluid pH and the risk of ruminal acidosis have not been reported for grazing dairy cows supplemented with FB during early lactation.

Comminution of forage starts physically, via oral processing during ingestion and continues with rumination of the regurgitated boli. Physical processing of ingesta aid microbial attachment and the chemical degradation of digesta. Microbial degradation of fibre declines *in vitro* and *in vivo* when ruminal pH is below 6.2 (Terry et al., 1969; De Veth and Kolver, 2001; Krajcarski-Hunt et al., 2002). The growth of fibrolytic and cellulolytic microbes decline under low pH conditions due to the increased energy needed to maintain intracellular pH. Reduced growth of cellulolytic and fibrolytic communities can lead to their washout from the rumen and reduces the degradation of structural carbohydrates (Russell and Wilson, 1996). The severity of SARA is defined by the duration below a certain pH, which in mild cases (pH <5.8 for > 180 min/d) can impair microbial metabolism, reducing the rate of rumen degradation and limiting milk production (Gozho et al., 2005). However, in severe cases, when pH declines below 5.6 for >180 min/d, long-term structural damage to the rumen epithelium can reduce the cow's ability to neutralise ruminal VFA, further increasing the risk of SARA. Long term structural damage to the rumen epithelium can also limit the absorption of VFA needed for metabolic and production purposes (Gozho et al., 2005; Zebeli et al., 2008).

Physical comminution of forage encourages microbial adhesion to ingesta and increases the outflow of digesta from the rumen. Mastication and chewing also promote salivation, which contains buffers that neutralise ruminal fluid's pH and encourage VFA absorption from the rumen (Mertens, 1997). While grazed herbage may require considerable oral processing and salivation before swallowing, the occurrence of moderate and severe SARA in grazing dairy herds is still widespread (Garrett et al., 1999; Bramley et al., 2013). Supplementation of readily digestible perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens L.*) herbage with FB will further dilute the dietary fibre fractions and its effectiveness. Recent indoor experiments by Waghorn et al. (2019) and Pacheco et al. (2020)

suggest that supplementation of perennial ryegrass herbage with moderate FB proportions will impair rumen function health, affecting performance. However, research on the effects of FB feeding in grazing studies is limited, and the impact on rumen degradation, ingestive and digestive processing of a perennial ryegrass-based diet during early-lactation has not been explored.

The objective of this study was to evaluate milk production, grazing behaviour, and digestive processes when a perennial ryegrass herbage is supplemented with moderate amounts (40% of total DMI) of harvested FB, during early-lactation. We hypothesised that supplementing perennial ryegrass even with moderate amounts of FB would increase the duration of time that ruminal fluid pH was below 5.8, impairing rumen function by reducing the ruminal digestion of perennial ryegrass and herbage intake by reducing grazing time.

## 5.3 Materials and Methods

The experiment was conducted during October and November of 2018 at the Lincoln University Research Dairy Farm (LURDF) in Canterbury, New Zealand (43°38'S, 172°27'E). All procedures were approved by the Lincoln University Animal Ethics Committee (AEC 2018-22). The current study is a continuation of previous research investigating the effect of FB supplementation on diet adaptation and milk production where cow management, experimental design, treatments, and management of forage and FB have been described previously (Chapter 4).

### 5.3.1 Animals, experimental design and treatments

Eight spring-calving, multiparous Holstein Friesian × Jersey dairy cows, fitted with a rumen cannula were stratified into two groups based on DIM (30 ± 11.6, mean ± SD), milk yield (27.4 ± 5.25, kg/d) and liveweight (482 ± 50.0, kg). Two treatments of either; HO, a herbage only control consisting of ~30 kg DM (above ground)/cow per d of an established perennial ryegrass and white clover sward, or FBH, which consisted of 6 kg DM of harvested FB bulb fed after morning milking and an allowance of 30 kg DM/cow per d (above ground) of the same herbage, were randomly allocated to each of the two groups. The cross-over experiment was conducted over two 20-d periods, separated by a 5-d (washout) period, during which all cows were returned to herbage to prevent first-order carry-over effects (Senn, 2002). Individual cows were the experimental unit as they grazed in individual paddocks (~ 60 $m^2$) and were fed FB individually. During each period, cows on the FBH diet were transitioned to target FB intake

(~40% of DMI) over 12 d by increasing the allocation + 0.5 kg DM/d following industry guidelines (Gibbs, 2014; Dalley et al., 2019). Cows were then adapted to the FBH diet between 12 to 17 d, and response variables were collected between 18 to 20 d. Cows were milked twice daily at approximately 0700 and 1600 h and had free access to fresh water from portable troughs at all times except during milking.

### 5.3.2 Management of fodder beet

The FB bulbs (cv. Enermax. D) were sourced from another farm and transported to the experimental site before the commencement of each period to maintain the bulb's chemical composition (Chapter 4). Fodder beet was harvested, and residual leaf was removed before transportation. Fodder beet bulbs were allocated to each cow in the FBH group individually, following the morning milking in plastic bins on a concrete feed pad. Cows were held on the feed pad for 2 h, or until completion of the FB meal before returning to a fresh paddock of herbage. Cows fed the HO diet returned to a new allocation of herbage following milking each morning. Daily refusals of FB were collected and weighed to estimate daily FB intake.

### 5.3.3 Herbage management

The sward allocated to all cows was dominated by perennial ryegrass (90.2 $\pm$ 3.41%; mean $\pm$ SE) and contained minor percentages of broad-leaved weeds (6.8 $\pm$ 2.42%, predominantly; *Rumex obtusifolius L.* and *Taraxacum officinalis*), white clover (3.4 $\pm$ 2.81 %), and dead material (2.9 $\pm$ 1.71%). Pastures (~3.0 ha each) were divided longitudinally into eighths using temporary fencing materials. The areas were used for grazing the 8 cows over 6-7 d by further dividing each strip into individual paddocks. Each paddock was grazed and fertilised with urea (~100 kg/ha) between 3-4 wk before the experiment. Pre- and post-grazing height were measured each day to estimate herbage mass and herbage allocation. Herbage mass was estimated using compressed height measured with a rising plate meter (RPM: Jenquip Ltd, New Zealand). The botanical and chemical composition of the sward was determined on day 17, before allocation to cows, by collecting random grab samples of herbage by hand (n = 5 per break) at grazing level (~ 3 cm above the ground). Approximately 20 rectangular quadrats (0.2 $m^2$) were collected from each paddock before the experiment. An additional two quadrats from each allocation were collected every third d of the experiment. Samples were washed, oven-dried, and weighed to determine DM content and develop a regression equation for estimating herbage mass using sward height, as reported previously in Chapter 4.

Daily intake of DM (kg/cow) and herbage were calculated from daily energy output and maintenance requirements minus the average daily loss of body condition (or plus the average daily gain of condition, assuming 1 BCS = 32.5 kg) during the experimental period (Roche et al., 2005). The energy calculations that were used have been reported previously by Holmes et al. (2002). The metabolizable energy requirements for maintenance of lactating dairy cows was 0.6 MJ/kg $BW^{0.75}$ (Holmes et al., 2002). The energy output from milk was calculated based on daily fat and protein content and total milk yield (kg), assuming a conversion efficiency of net energy to milk energy of 65%, and the efficiency of liveweight mobilization was 80%.

$$\mathrm{DMI} = \frac{\left((\text{Lactation energy} + \text{maintenance energy} + \text{walking energy}) - \text{BC loss} + \text{BC gain}\right) - (\text{FB intake} \times \text{FB ME})}{\text{Herbage ME concentration}} \quad (5.1)$$

Cows were situated adjacent to the milking shed on flat terrain and were assumed to walk approximately 1 km/d while walking to the milking parlour and grazing [liveweight $\times$ (0.026 $\times$ Horizontal km)/ $k_m$]. Where $k_m$ is the efficiency of ME utilization calculated by ME concentration of the diet [(ME $\times$ 0.02) + 0.5] (Nicol and Brookes, 2007). The calculated ME intake from FB was subtracted from the total apparent energy intake and divided by the ME concentration of herbage to calculate DMI.

### 5.3.4 Plant sub-sampling and analyses

Hand grab samples of herbage were bulked, homogenised, and separated into thirds to determine DM% (oven-dried at 60℃ for 48 hours), chemical and botanical components. Botanical components were sorted (perennial ryegrass, white clover, weeds, and dead material) and oven-dried to calculate relative abundance in the sward. The third sample was frozen (-20℃) and stored until freeze-dried, ground through a 1mm sieve (ZM200 Retsch GmbH; Haan, Germany) and chemical components (CP, ADF, NDF, and OM) were determined by near-infrared spectroscopy (NIRS. Model: FOSS NIRS Systems 5000, Maryland USA). Three bulbs of FB were randomly selected from the face of the stack to analyse DM and chemical composition. Briefly, FB bulbs were cut into four equal sections by halving the bulb lengthways and then halving lengthways again. Each section was minced separately using an electric hand blender. One section was weighed and oven-dried (100℃) over 72 h, and the second was frozen (-20℃) and stored until freeze-dried, ground through a 1 mm sieve, and analysed for chemical components (CP, ADF, NDF, and OM) using NIRS. Calibration equations for predicting WSC, CP, ADF, NDF, and OM of FB were developed previously on FB bulb samples. The R-

squared values for CP, OM, WSC, NDF, and ADF of both FB and perennial ryegrass herbage were all above 0.90, and all samples were within the calibration range. The metabolizable energy content of forages was calculated using the modified ADF (MADF) method, ME (MJ/kg DM) = 14.55-0.015×MADF (CSIRO, 2007).

### 5.3.5 Animal measurement and sample analysis

Liveweight, and milk yield (kg) were measured automatically at each milking (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden). The BCS of each cow was assessed and recorded by a certified BCS assessor (DairyNZ Ltd.) on d 0 and d 20 of each experimental period using a 1-10 scale (Roche et al., 2004a). Milk samples from individual cows were collected using in-line milk meters from two consecutive milkings (PM of d 19 and AM of d 20) to determine the proportion and yield of protein, fat, lactose, and MS, which was analysed by the laboratory of Livestock Improvement Corporation Ltd (Christchurch, New Zealand) using Milkoscan (Foss Electric, Hillerod, Denmark). A skimmed milk sample was frozen at -20℃ until analysed for MUN by the enzymatic kinetic method using Randox RX Daytona analyses (clinical assay kit: UR 3825, Randox Rx Daytona, Randox Laboratories, Ltd, United Kingdom).

### 5.3.6 Ruminal pH and rumen sampling

The pH of ruminal fluid was measured every 10 min using a wireless bolus (SmaXtec animal care GmbH, Austria) as described previously in Chapter 4. On day 20 of each period, cows were herded to the yards for rumen sampling every 4 h, a procedure which took approximately 40 min between leaving and returning to herbage areas. Once in the yards, random hand grab samples of rumen digesta were collected from the ventral sac of the rumen. Digesta was filtered through 2 layers of an open-weave cloth (Superwipes, Clorox, Australia) into two 2 mL microtubules to measure ammonia ($NH_3$; acidified with 6 M sulphuric acid) and VFA concentration and were stored at -20℃ until assessed. The concentration of VFA was determined by gas chromatography using an SGE BP21 30 m x 530 µm x 1.0 µm wide-bore capillary column using an autosampler (AOC-20i) fitted to a Shimadzu GC-2010 gas chromatograph following the method of (Chen and Lifschitz, 1989). Ammonia and L-lactate concentrations of rumen fluid were determined enzymatically using commercially available kits from Randox Daytona ($NH_3$ clinical assay kit: AM 3979, L-lactate clinical assay kit: LC 3985).

### 5.3.7 *In sacco* incubation

Samples of perennial ryegrass and FB bulb were incubated in separate Dacron bags (10 × 15 cm with 50µm pore size) in cows on the FBH treatment, while only samples of ryegrass were incubated in cows on the HO treatment. Before incubation, perennial ryegrass was collected in the morning, mixed, sub-sampled, and weighed into Dacron bags. One sub-sample was used to determine DM%, and a second sub-sample was stored at -20°C until NIRS determined chemical components. Fodder beet bulbs were processed as described previously. Samples of FB bulb and perennial ryegrass were separately blended to < 5 mm (to imitate mastication) and incubated *in sacco*, following procedures adapted from Barrell et al. (2000). Approximately 40-60 g of fresh FB or perennial ryegrass was weighed into each Dacron bag, cable tied to a galvanised chain, and frozen at -20 °C until incubated. Dacron bags were removed after 0, 4, 8, 12, 16, and 20 h of incubation. Each chain could fit within a 5 L bucket to reduce the effect on rumen fill on DMI. Each cow received one metal chain anchor suspended inside the rumen at 0400 h on day 20 of each period. The collection of Dacron bags from the rumen occurred simultaneously as the collection of rumen digesta samples at 0400, 0800, 1200, 1600; 2000, and 2400 h. Upon removal, rumen bags were submerged in ice water, and excess digesta was removed, then bags machine rinsed, using a cold wash cycle for 10 minutes and oven-dried at 60ºC for 72 h to calculate residual DM.

### 5.3.8 Rumen bailing and particle distribution

The rumen contents were bailed at ~0000 h on day 20, and the digesta was removed from each cow and placed into individual large 50-80L bins to estimate the outflow of solid digesta. Rumen bailing took place at midnight following the method of Taweel et al. (2005). Cows were fasted following the dusk grazing bout's completion to prevent carry-over effects (Gregorini et al., 2009a). During rumen bailing, one subsample was collected from approximately every 20 hand grab samples, including liquid contents, to gather a representative sample of the liquid and solid components. Once empty, the rumen digesta was weighed and recorded (including the bulk grab sample) and sequentially returned to the rumen. Cows were fasted indoors until ~0930 h, after the morning milking, when the rumen bailing procedure was repeated before the cows were returned to their paddocks. The grab samples collected from each bailing session were filtered through two layers of an open weave cloth (Superwipes, Clorox, Australia) to separate liquid and solid fractions and weighed. Samples of the solid fraction were collected to determine DM% (by oven drying 100ºC for 72 h), chemical

components (OM, NDF, ADF, and ADL), and particle size (by wet sieving). A sample of solid rumen digesta was freeze-dried and ground to pass through a 1 mm sieve, and the proportion of DM and OM was determined by oven drying at 100 ºC and ignition at 550 ºC. The NDF and ADF content of rumen digesta were analysed following previous methods (Van Soest et al., 1991a). The ADL component was determined following the ADF step by mixing the residual pellet with 72% sulphuric acid (Miller, 2008). Concentrations of ADF and ADL were determined from separate samples, and the content of both NDF and ADF is expressed as residual ash.

An estimate of the ruminal NDF outflow and fractional degradation rate was calculated using the logarithmic transformation of the below Equation, as described by Taweel et al. (2006).

$$R_t = R_o \times e^{-CL \times t} \quad (5.2)$$

Where $R_t$ is the amount of NDF present at the first bailing session at midnight, $R_o$ is the amount of NDF remaining at the second bailing session, the next morning (0930 h), CL is the fractional outflow of NDF (% /hr), and *t* is the time between the 2 evacuations in h. The fractional ruminal outflow of NDF was corrected using acid detergent lignin (ADL), assuming that ADL is rumen undegradable and removed from the rumen via passage through the ruminal-reticular orifice. Factional degradation of NDF may be underestimated using this method as ADL may pass through the rumen at a rate greater than NDF (Tamminga et al., 1989). The suitability for using ADL as an internal marker has been previously addressed (Taweel et al., 2005). The pool of rumen fermentation end-products was determined by multiplying individual VFA concentration by the rumen liquid pool.

Particle size fractions of rumen digesta were determined by wet sieving using the method of Waghorn et al. (1986). One sample (~30 g) was weighed and oven-dried 100ºC for 48 hours to determine DM%. Samples of the rumen digesta collected from each cow at each rumen bailing were duplicated and washed for 5 minutes under a recirculating flow (Waghorn et al., 1986). Digesta was passed through six metal sieves with apertures of 4, 2, 1, 0.5, 0.25 mm, and 75 μm, in respective order. Following the five min wash period, the water flow was turned off, and the contents of each sieve were transferred to filter paper oven-dried at 100º C, over 24 hours to measure dry weight. The DM proportion on each sieve was calculated and soluble fractions were calculated as the difference between pre- and post-sieving DM weight. Concentrations of particle sizes were multiplied by the DM pool of rumen digesta at each

bailing session to determine the DM pool of large (> 2mm) medium (< 2 mm and > 0.5 mm) and small (< 0.5 mm) particles.

### 5.3.9 Grazing behaviour

On day 16, once FBH cows had consumed the maximum FB allocation for at least four consecutive days, a jaw movement recorder (UltraSound Advice, London, UK) was fitted to each cow to record individual jaw movements over 24 hours. Jaw recorders consisted of a transducer that formed a noseband that recorded the electrical resistance as the jaw opened and closed to a micro-computer containing a data logger, a memory card, and a battery (Rutter et al., 1997). Prehension, mastication, and individual boli were differentiated automatically using the 'GRAZE' software, which automatically analyses jaw movements into bite data (Rutter, 1998). Length of grazing, rumination, or FB bouts was determined by manually analyzing jaw amplitude and identifying each bout's start and end. The minimum inter-bout length required between grazing bouts was 420 seconds (Rutter et al., 1997). Grazing data included the period of grazing, rumination, idling, and eating supplement and counts of prehension, mastication, and rumination boli while grazing, ruminating, or eating FB.

### 5.3.10 Statistical Analysis

The statistical analyses compared the two treatments using a mixed model ANOVA with the lme function of the 'lme4' package (Pinheiro et al., 2018) in R (R Core Team, 2018, v. 3.4.4.). For discrete data (e.g., the number of mastications, prehensions, and the number of rumination boli per d), a generalized linear mixed model with a Poisson distribution using the 'glmer' function of the 'nlme' package was used to conduct statistical analyses. The remaining data (continuous) was analysed using a linear mixed-effects model. In both models, the individual cow was the experimental unit, diet, time (when appropriate), and period were fixed effects, and individual cow nested within the day was the random effect. Apparent rumen DM disappearance was measured over 20 h of incubation, which was not sufficient for complete degradation of fermentable material and did not provide enough time points to determine the disappearance rate using the model outlined by Ørskov and McDonald (1979). Therefore, rumen DM disappearance *in sacco* was considered as a factorial arrangement and analysed using a mixed-effects ANOVA where, period and the interaction between plant (FBB, FBH ryegrass, and HO ryegrass) and incubation time were fixed effects while the cow was

treated as a random effect. For all variables, the default, unstructured covariance structure of the 'nlme' package was used as it produced the smallest Akaike information criterion when compared with other covariance structures. Least means squares were determined using the 'emmeans' package (Lenth, 2018) of R, upon the significance of the ANOVA. Pairwise contrasts were determined using Tukey's method in the 'emmeans' package to separate the means of significant interactions ($P < 0.05$). Differences were declared significant if $P \leq 0.05$ and tendencies were $0.05 < P \leq 0.10$.

## 5.4 Results

During each period, one cow from the FBH group developed SARA symptoms on day 10 of adaptation in period 1 and day 15 of adaptation during period 2 (pH < 5.5 for 110 and 240 min/d, period 1 and 2, respectively). The allocation of FB to the two affected individuals was reduced to 3 kg DM/d for the remainder of the experiment. Data collected for the two affected cows were included in statistical analyses as ruminal pH stabilised without intervention, a characteristic of SARA.

### 5.4.1 Intake and milk production

Chemical composition of herbage fed to either FBH or HO treatments were not different ($P > 0.05$; Table 5.1). Fodder beet bulb contained lower proportions of NDF, ADF, CP, and N, but greater proportions of OM and WSC than herbage ($P < 0.001$).

Estimated DMI from energy output, liveweight, and milk yield are presented in Table 5.2. The average herbage allocation over both periods was similar between treatments. Fodder beet bulb represented 38% of daily DMI for the FBH treatment. The ME required (182 and 186 MJ ME/d, FBH, and HO, respectively) and estimated DMI (15.6 and 16.2 kg DM/d, FBH, and HO, respectively) were similar between treatments although, herbage intake declined 38% with the FBH diet. Milk yield was not different ($P > 0.10$) between treatments. The FBH diet did not ($P > 0.10$) alter the fat or protein proportions or yield in milk. The FBH diet reduced percentage ($P = 0.01$), but not ($P = 0.24$) yield of lactose (Table 5.2).

Table 5.1. Average pre and post-grazing mass and chemical composition[2] (%) of fodder beet (FB) and herbage fed as a herbage only diet (HO) or herbage fed to cows supplemented with FB (FBH).

| Item | Period 1 | | | | Period 2 | | | | *P*-Value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Herbage | | FB | | Herbage | | FB | | | |
| | HO | FBH | bulb | SE[1] | HO | FBH | Bulb | SE | Plant | Period |
| Pre-grazing (kg DM/ha) | 5497 | 5453 | - | 86 | 3478 | 3596 | - | 61 | 0.14 | <0.001 |
| Post-grazing (kg DM/ha) | 2823 | 3050 | - | 68 | 1953 | 2277 | - | 59 | <0.001 | <0.001 |
| Area ($m^2$/cow/d) | 53.6 | 52.2 | - | 1.06 | 76.3 | 73.4 | - | 1.71 | 0.18 | <0.001 |
| Chemical composition[3] | | | | | | | | | | |
| DM (% DM) | $14.7^{c}$ | $14.2^{c}$ | $12.7^{a}$ | 0.36 | $17.5^{d}$ | $18.1^{d}$ | $20.7^{b}$ | 0.60 | <0.001 | <0.001 |
| OM (% DM) | $91.5^{b}$ | $91.4^{b}$ | $94.2^{a}$ | 0.26 | $91.8^{b}$ | $91.7^{b}$ | $93.7^{a}$ | 0.29 | <0.001 | 0.75 |
| ADF (% DM) | $21.0^{c}$ | $21.2^{c}$ | $7.81^{a}$ | 0.123 | $23.3^{d}$ | $23.6^{d}$ | $8.15^{b}$ | 0.130 | <0.001 | <0.001 |
| NDF (% DM) | $36.6^{d}$ | $37.7^{c}$ | $13.0^{a}$ | 0.185 | $41.7^{e}$ | $41.8^{e}$ | $14.0^{b}$ | 0.241 | <0.001 | <0.001 |
| WSC (% DM) | $21.1^{b}$ | $20.6^{b}$ | $63.9^{a}$ | 0.39 | $20.5^{b}$ | $20.2^{b}$ | $59.4^{a}$ | 0.41 | <0.001 | 0.15 |
| CP (% DM) | 15.6 | 16.0 | 8.23 | 0.27 | 15.7 | $15.5^{b}$ | $9.39^{a}$ | 0.37 | <0.001 | 0.29 |
| ME (MJ/kg DM) | $11.1^{a}$ | $11.1^{a}$ | $13.5^{b}$ | 0.05 | $10.8^{c}$ | $10.8^{c}$ | $13.2^{b}$ | 0.07 | <0.001 | <0.001 |
| Ether extract (% DM) | $2.30^{c}$ | $2.72^{b}$ | $0.59^{a}$ | 0.084 | $2.12^{c}$ | $2.54^{b}$ | $0.40^{a}$ | 0.088 | <0.001 | 0.13 |

[a-e] Means within rows with different superscripts are significantly different (P<0.05). [1] SE: Standard error of estimated marginal means. [2] Herbage allocation above ground. [3] ADF: Acid detergent fibre, NDF: neutral detergent fibre, WSC: water-soluble carbohydrate, OM: organic matter, CP: crude protein, N: nitrogen, ME: metabolizable energy.

### 5.4.2 Ruminal pools of digesta and fermentation products

The diurnal variation of ruminal fluid pH on day 20 is displayed in Figure 5.1. There was a diet effect between 0400 h and 1300 h ($P < 0.001$). Ruminal pH declined following the allocation of either herbage or FB in the morning. The FBH diet caused the pH of the ruminal fluid to decline to 5.6 by 1100 h compared with HO, in which pH declined to 6.0 by midday ($P < 0.001$). The pH of ruminal fluid measured in cows fed FBH remained below 5.8 between 0930 h to 1200 h each day, while ruminal fluid pH in cows fed HO remained above 5.8.

Solid, liquid, and fibre components of rumen digesta are presented in Table 5.3. There were no interactions between diet and time of rumen bailing on the solid or liquid proportion of digesta ($P > 0.10$). Total digesta weight declined 38% between the first and second rumen bailing ($P < 0.001$). The liquid and solid proportions of digesta were similar between treatments ($P = 0.22$, $P = 0.43$, respectively). The solid and liquid pools of digesta declined by

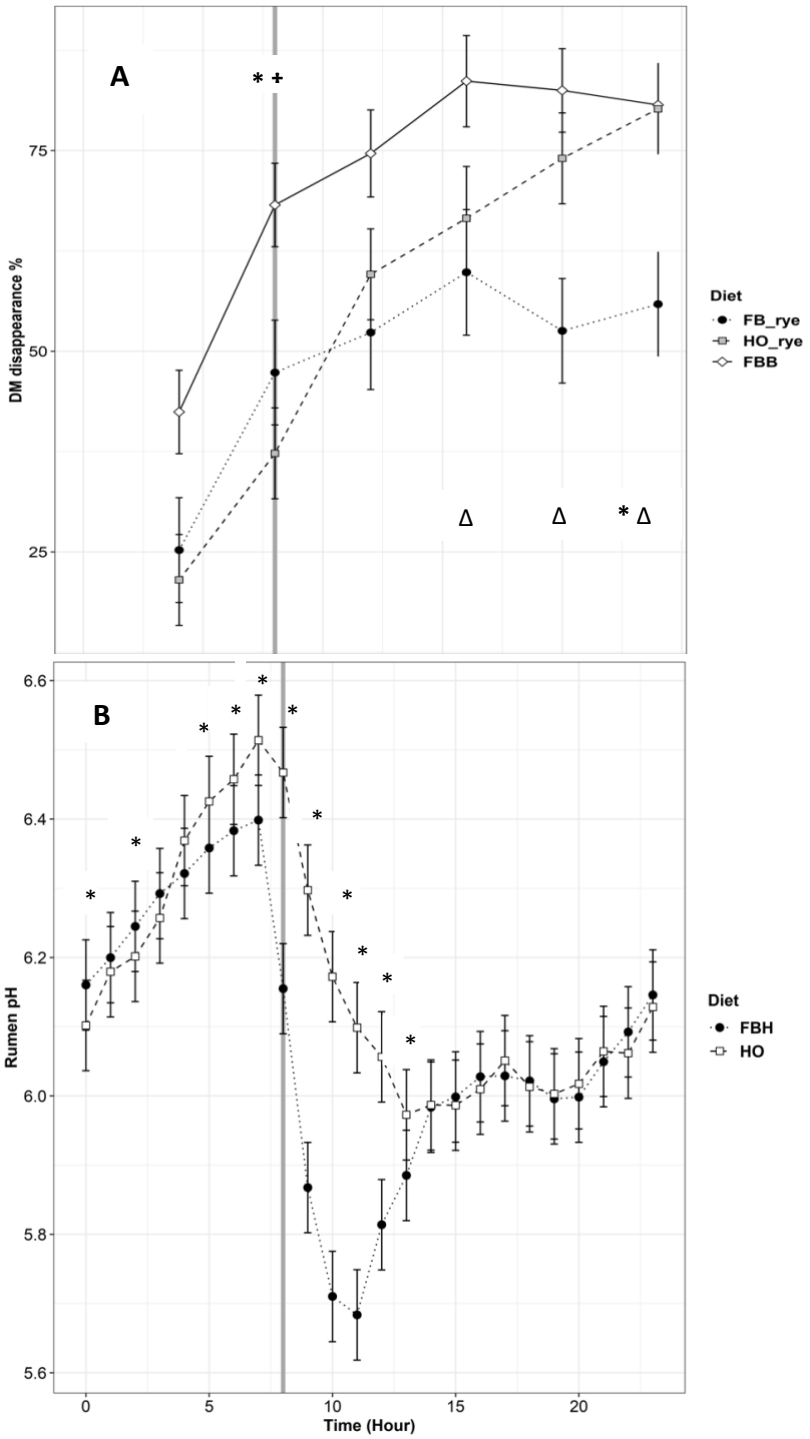


Figure 5.1. (A) Apparent DM disappearance of ryegrass incubated in cows fed HO (HO_rye) or ryegrass (FBH_rye), and fodder beet bulb (FBB) incubated in cows fed FBH. (B) Diurnal variation of ruminal fluid pH from cows fed FBB and herbage (FBH) or herbage only (HO). Vertical reference lines indicate the time of either FB or herbage allocation. In A, significant differences ($P < 0.05$) between FB_rye and HO rye are indicated by *, differences between FBB and HO_rye are indicated by †, and differences between FBB_rye and FBB are indicated by Δ. In B, * is used to indicate that the effect of diet is significant ($P < 0.05$).

40% and 38%, respectively, between the first and second rumen bailing (Table 5.3). The FBH diet tended to increase the pool of NDF at the second rumen bailing session ($P$ = 0.08) compared with HO but did not alter the pool of DM, OM, ADF, or ADL (P > 0.10). The ruminal outflow rate of NDF (48.6 and 60.3 ± 4.88 g/h, $P$ = 0.81, FBH and HO, respectively) and ADL (9.88 and 9.91 ± 1.98 g/h, $P$ = 0.99, FBH and HO, respectively) were similar between treatments. The effect of dietary treatment on the fractional rumen degradation rate of NDF was not significant ($P$ = 0.71), averaging 38.7 and 40.4 g NDF/hr for cows fed FBH and HO, respectively.

The concentrations of fermentation-end products have been reported previously (Chapter 4), and only a brief description of VFA concentrations are reported. The FBH diet reduced acetate concentrations by 7%, isovalerate by 20%, and isobutyrate by 15% compared with HO ($P$ < 0.01).The FBH diet increased butyrate concentrations by 21% ($P$ = 0.006), valerate by 44% ($P$ < 0.001) and caproate by 33% ($P$ <0.001) compared with HO. The FBH diet reduced the volume of ammonia (33%, $P$ = 0.04), acetate (10%, $P$ < 0.001), isobutyrate (17%, $P$ < 0.001) and isovalerate (22.2%, $P$ < 0.001) compared with the cows fed the HO diet (Table 5.3). The FBH diet also increased the pool of butyrate (19%, $P$ = 0.05), valerate (42%, $P$ <0.001) and caproate (31%, $P$ < 0.001) in the rumen compared with the HO treatment. The pool of lipogenic (acetate + butyrate) VFA declined 18% with the FBH diet at the second rumen bailing session, compared with HO ($P$ < 0.01). The pool of glucogenic VFA (propionate and lactate) was not different ($P$ = 0.47). However, diet by time interactions were significant ($P$ = 0.014) for the ruminal pool of total volatile fatty acid (TVFA), which declined 15% with the FBH treatment at the second rumen bailing session, compared with HO (Table 5.3).

Table 5.2. Estimated intake of herbage (HI) and fodder beet (FB), total dry matter intake (DMI), and yield of whole milk (kg/d) and milk constituents of cows fed either fodder beet bulb + herbage (FBH) or a herbage only (HO) diet.

| | Diet | | | *P*-Value |
|---|---|---|---|---|
| **Item** | HO | FBH | SE[1] | Diet |
| BCS (1-10 scale) | 4.3 | 4.1 | 0.14 | 0.51 |
| BCS change | 0.1 | 0.3 | 0.20 | 0.65 |
| Liveweight (kg) | 497 | 499 | 6.4 | 0.45 |
| ME required (MJ/d) | 180 | 186 | 9.8 | 0.14 |
| HI[2](kg DM/d) | 16.2 | 9.9 | 0.89 | <0.001 |
| FB refusal (kg DM) | 0 | 0.99 | 0.17 | <0.001 |
| FB Intake (kg DM) | 0 | 5.79 | 0.15 | <0.001 |
| Estimated DMI (kg/d) | 16.2 | 15.6 | 0.88 | 0.17 |
| Milk components | | | | |
| Fat (%) | 5.09 | 5.05 | 0.364 | 0.92 |
| Protein (%) | 3.87 | 4.03 | 0.164 | 0.10 |
| Lactose (%) | 5.17 | 5.07 | 0.066 | 0.01 |
| MS[5] (%) | 8.96 | 9.07 | 0.471 | 0.82 |
| Yield of milk components | | | | |
| Fat (kg/d) | 1.22 | 1.21 | 0.086 | 0.85 |
| Protein (kg/d) | 0.93 | 0.95 | 0.045 | 0.55 |
| Lactose (kg/d) | 1.26 | 1.22 | 0.087 | 0.24 |
| MS[3] (kg/d) | 2.15 | 2.16 | 0.116 | 0.99 |
| Milk (kg/d) | 23.6 | 23.4 | 1.26 | 0.81 |

[1]SE: standard error of estimated marginal means.

[2] Herbage intake estimated from energy output in milk and maintenance requirements – the ME received from FB/ME content of herbage.

[3]Milk Solids (fat + protein)

Table 5.3. Average total weight and percentage of solid, liquid organic matter and fibre components of rumen digesta and pool of fermentation-end products collected by rumen bailing at midnight (0000 h) and morning (0930 h) from cows fed either a fodder beet bulb and herbage (FBH) or herbage only (HO) diet.

| | Midnight | | | Morning | | | *P*-Value | | |
|---|---|---|---|---|---|---|---|---|---|
| **Item** | HO | FBH | SE[1] | HO | FBH | SE | Diet | Time | D×T[2] |
| Total rumen weight (kg) | 88.4[a] | 91.0[a] | 3.39 | 55.1[b] | 56.6[b] | 3.39 | 0.14 | <0.001 | 0.66 |
| Rumen solid (kg DM) | 8.82 | 8.48 | 0.473 | 4.94 | 5.45 | 0.473 | 0.82 | <0.001 | 0.23 |
| Rumen liquid (kg) | 79.5 | 82.5 | 3.05 | 50.2 | 51.5 | 3.05 | 0.12 | <0.001 | 0.42 |
| NDF (kg) | 4.60[a] | 4.73[a] | 0.467 | 2.96[b] | 3.40[cb] | 0.239 | 0.06 | 0.004 | 0.08 |
| ADF (kg) | 2.64[a] | 2.67[a] | 0.229 | 1.61[b] | 1.82[b] | 0.116 | 0.22 | <0.001 | 0.11 |
| ADL (kg) | 0.40[a] | 0.42[a] | 0.038 | 0.29[b] | 0.32[b] | 0.027 | 0.44 | <0.001 | 0.62 |
| OM (kg) | 7.85[a] | 7.57[a] | 0.657 | 4.49[b] | 4.99[b] | 0.306 | 0.75 | <0.001 | 0.14 |
| **Fermentation products** | | | | | | | | | |
| $NH_3$ (mole) | 0.42[a] | 0.28[b] | 0.045 | 0.14[c] | 0.06[d] | 0.012 | 0.042 | <0.001 | 0.03 |
| Acetate (mole) | 5.73[a] | 5.15[b] | 0.20 | 2.94[c] | 2.38[d] | 0.078 | <0.001 | <0.001 | 0.008 |
| Butyrate (mole) | 1.28[b] | 1.58[a] | 0.017 | 0.45[c] | 0.40[c] | 0.064 | 0.05 | <0.001 | 0.026 |
| Propionate (mole) | 1.96[a] | 1.87[a] | 0.079 | 0.76[b] | 0.73[b] | 0.025 | 0.36 | <0.001 | 0.80 |
| Lactate (mmol) | 0.87 | 1.26 | 0.342 | 0.21 | 0.19 | 0.065 | 0.59 | 0.015 | 0.83 |
| Valerate (mole) | 0.13[a] | 0.22[a] | 0.164 | 0.04[c] | 0.07[b] | 0.003 | <0.001 | 0.001 | 0.016 |
| Caproate (mole) | 0.050[a] | 0.072[a] | 0.0036 | 0.021[b] | 0.025[b] | 0.0013 | <0.001 | <0.001 | 0.80 |
| NG:G ratio[7] | 2.54[a] | 2.62[a] | 0.193 | 3.83[b] | 3.33[b] | 0.268 | 0.53 | 0.003 | 0.47 |
| TVFA[8] (mole) | 9.33[a] | 9.03[a] | 0.399 | 4.30[b] | 3.67[c] | 0.155 | 0.08 | <0.001 | 0.014 |

[a-d] Different superscripts within rows are different ($P < 0.05$). [1] SE: standard error of estimated marginal means. [2] Diet by sampling time interaction. [3] Acid detergent lignin. [4] Acid detergent fibre. [5] Neutral detergent fibre. [6] Organic matter. [7] Non-glucogenic (acetate + butyrate) to glucogenic (propionate + lactate) ratio. [8] Total volatile fatty acid.

Diet by time, interactions for lactic acid concentrations or pool in the ruminal fluid were not significant ($P > 0.10$). While time by treatment interaction for the ruminal concentration of TVFA was not significant, a significant interaction between diet and time of rumen bailing was detected for the pool of TVFA in ruminal fluid ($P < 0.001$).

### 5.4.3 Particulate pools turnover and dry matter disappearance

Particle DM fractions and particulate pools of rumen digesta are presented in Table 5.4. The proportion of large and medium-sized particles increased, and the proportion of small particles declined ($P < 0.001$) between the first and second rumen bailing. The DM pool of large ($P < 0.001$) and small ($P < 0.001$) particle sizes also declined while medium pools tended ($P = 0.06$) to decline between the first and second rumen bailing. There was an interaction between diet and time of rumen bailing for DM fractions retained on small, medium, and large sieve sizes (Table 5.4). Between the first and second ruminal bailing, the FBH diet increased the DM fraction of large particles by 25% ($P = 0.01$), and the DM pool of large particles increased 27% ($P = 0.003$) compared with HO. The proportion of small particles declined between the first and second rumen bailing ($P < 0.001$), while the FBH treatment increased the disappearance of small particles between the first and second rumen bailing by 12% compared with HO ($P = 0.04$).

Results of *in sacco* DM disappearance are presented in Figure 5.1. Diet by time interactions were significant ($P < 0.001$). The FBH diet reduced the extent of DM disappearance of perennial ryegrass by 24% following 20 h incubation ($P < 0.001$). Plant by time interactions was detected at all-time points for DM disappearance of herbage (FBH and HO) and FB bulb. By 16 h of incubation, the FBH diet tended to reduce ($P = 0.06$) the DM disappearance of perennial ryegrass herbage and was significantly less ($P < 0.01$) by 20 h of incubation than perennial ryegrass incubated in cows fed the HO diet.

### 5.4.4 Grazing behaviour

Time spent grazing, ruminating, idling, and consumption of FB are presented in Table 5.5. Total eating time was 9.16 and 8.42 h/d for HO and FBH, respectively. The time spent eating FB

represented 7% of total daily activity or 16% of eating activity. The FBH diet reduced the time spent grazing by 21% ($P < 0.001$) and increased rumination and idling time by 16% ($P = 0.03$) and 31% ($P = 0.02$), respectively, compared with the HO treatment (Table 5.5). While FBH increased rumination time, the number of boli regurgitated each day was similar to the HO treatment ($P = 0.28$). However, cows fed FBH regurgitated an additional 104 boli/d compared with those fed HO. The FBH diet increased total mastication jaw movements per d by 5.5% ($P < 0.001$) compared with HO. The mastication of FB represented 14.6% of total mastications per d. The FBH diet increased chewing frequency while ruminating by 38% compared with HO ($P < 0.001$). In the FBH treatment, the number of mastications while grazing was 38% less than HO ($P < 0.001$). The FBH treatment did not alter the number of grazing, ruminating, or idling bouts compared with the HO diet (Table 5.5; $P > 0.10$). However, the duration of grazing bouts declined by 21% ($P < 0.001$) when cows were fed the FBH diet. There were three bouts during the FB meal, which averaged 42 minutes each. The average number of mastications during each FB eating bout was equal to the number of chews experienced during a rumination bout (Table 5.5). Furthermore, feeding cows FB reduced the mean number of bites during each grazing bout by 46% ($P < 0.001$) and also reduced the number of mastications per grazing bout by 51% ($P < 0.001$) compared with HO (Table 5.5).

## 5.5 Discussion

We hypothesised that supplementation of perennial ryegrass with FB would increase the duration of low ruminal fluid pH (pH < 5.8), impairing the ruminal digestion of perennial ryegrass and herbage intake by reducing grazing time. Based on our ruminal fermentation, particle comminution, and grazing behaviour results, we accept our hypothesis.

Table 5.4. The percentage of particle size and the total pool of particles in rumen digesta collected from the rumen of cows fed either a fodder beet bulb and herbage (FBH) or herbage only diet (HO) at midnight (0000 h) and following fasting the next morning (0930 h)

| | Midnight | | | Morning | | | *P*-Value | | |
|---|---|---|---|---|---|---|---|---|---|
| **Particle fractions** | HO | FBH | SE[1] | HO | FBH | SE[1] | Diet | Time | D×T[2] |
| ≥ 2mm (%) | 25.3[a] | 22.9[a] | 1.31 | 26.0[a] | 32.5[c] | 1.52 | 0.10 | <0.001 | 0.01 |
| < 2 and ≥ 0.5 mm (%) | 15.0[a] | 17.5[b] | 0.70 | 23.7[c] | 23.7[c] | 0.96 | 0.17 | <0.001 | 0.02 |
| < 0.5mm (%) | 58.4[a] | 59.4[a] | 2.19 | 49.1[b] | 43.1[c] | 1.64 | 0.16 | <0.001 | 0.04 |
| **Particle pool** | | | | | | | | | |
| ≥ 2mm (kg) | 2.1[a] | 2.01[a] | 0.138 | 1.31[c] | 1.8[b] | 0.184 | 0.11 | <0.001 | 0.003 |
| <2 and ≥ 0.5 (kg) | 1.3[b] | 1.52[a] | 0.103 | 1.17[b] | 1.32[ab] | 0.086 | 0.03 | 0.06 | 0.87 |
| < 0.5 mm (kg) | 5.01[a] | 5.25[a] | 0.301 | 2.41[b] | 2.3[b] | 0.118 | 0.77 | <0.001 | 0.34 |

[a-c] different superscripts within rows differ (P<0.05)

*P<0.05, **P<0.01, ***P<0.001

[1]SE: standard error of estimated marginal means.

[2] Diet by time interactions

### 5.5.1 Milk production, rumen fermentation patterns and pH

While the FBH diet was hypercaloric, compared with HO, cows fed FBH consumed a similar amount of ME than HO cows. However, the FBH treatment did not benefit milk production, consistent with previous studies where herbage was supplemented with FB bulb (Waghorn et al., 2019; Pacheco et al., 2020). It is important to note that the estimation of herbage mass, using calibration equations, underestimated the herbage mass available to the FBH treatment due to the high herbage mass offered to all treatments, which increased trampling and selective grazing of the FBH cows. Evidence of this error is provided by the similar milk production and energy requirements calculated between treatments.

In agreement with our hypothesis, the reduction of ruminal fluid pH between 4 AM and 1 PM in cows fed FBH may have limited the milk response to FB supplement. Low ruminal pH was caused by the accumulation of VFA that occurred following the FB meal. It is important to note that other than the two cows which developed SARA, the low ruminal fluid pH of cows fed FBH was not indicative of SARA but may have reduced pH to sub-optimal levels for rumen microbial activity (De Veth and Kolver, 2001; Krajcarski-Hunt et al., 2002). In Chapter 4, the time-dependent changes to rumen fermentation during dietary adaptation to FB were evaluated, and it was that individual cows might require a more gradual and more prolonged adaptation to FB to prevent the decline of ruminal fluid pH. The significant decline of ruminal acetate and TVFA pools following the fasting period, the reduction of *in sacco* DM

disappearance of perennial ryegrass, and the reduced comminution of large particles in cows fed FBH further support the previous and the current hypotheses.

While the risk of SARA has been attributed to feeding FB management errors related to FB yield estimation, individual accessibility to FB, or poor transitioning methods (Gibbs, 2014), 25% of cows still experienced SARA towards the end of the transitioning period, even though they were under controlled individual feeding conditions. Previous studies have reported acute and SARA occurrence when FB is fed > 40% of DMI during late-lactation (Dalley et al., 2019; Waghorn et al., 2019; Pacheco et al., 2020). Risk of SARA — at similar feeding proportions of FB — may be enhanced during early lactation both by the reduced absorptive capacity of the rumen and the reduced secretion of saliva, which contrast with the increased energy demands experienced during early and peak lactation (Cassida and Stokes, 1996; Penner et al., 2007; Dohme et al., 2008). While at the group level, milk production from the FBH treatment was not reduced compared with HO, individuals who experienced an extended duration of low pH below 5.5 are at risk of developing ruminitis, and parakeratosis (Gäbel et al., 2002; Krajcarski-Hunt et al., 2002), increased oxidative stress and suppressed immune function (Bull et al., 1965; Gozho et al., 2005; Guo et al., 2013). Our results indicate a high variation of response between individuals when spring herbage is supplemented with FB. Therefore, further research of such outcomes and incidence or SARA-related disorders at the herd-scale are still required.

### 5.5.2 Rumen degradation and oral processing

Our results indicate that the decline of ruminal fluid pH due to feeding FB reduced the rumen's fibrolytic activity. The growth of fibrolytic microbes is impaired when pH is < 5.8, while damage to epithelial tissue can occur when pH is < 5.6 (Zebeli et al., 2012b). De Veth and Kolver (2001) reported that DM digestibility declined 16.1%, and apparent NDF digestibility declined 11.7% when the ruminal fluid pH was below 5.8. However, the FBH diet did not alter the rate of ruminal NDF or ADL outflow. While the observed rate of NDF outflow was similar to previous reports (49.5 versus 56 g/h), the rate of ADL turnover was 3-fold less in the current experiment (10 versus 30 g/hr), and the fractional NDF degradation rate was greater than previously observed for mid-lactation dairy cows which grazed a ryegrass herbage in summer, 39.6 versus 25 g NDF/hr (Taweel et al., 2005). However, the ruminal pool of ADL observed in the present study was similar to that reported by Taweel et al. (2005), which indicates differences may be due to the physiological state of the experimental animals and ryegrass herbage used, as the

Table 5.5. Grazing behaviour, mean duration of daily activity, oral processing (mastication, prehension, and boli), and bout length of cows fed a herbage and fodder beet bulb (FBH) or herbage only diet (HO).

| | Diet | | | *P*-Value |
|---|---|---|---|---|
| **Activity** | FBH | HO | SE[1] | Diet |
| Rumination (min/d) | 539 | 453 | 30 | 0.03 |
| Grazing (min/d) | 440 | 556 | 18 | <0.001 |
| Supplement (min/d) | 82.0 | 0 | 8.0 | |
| Total eating (hr/d) | 8.74 | 9.69 | 0.33 | 0.09 |
| Idle (min/d) | 309 | 213 | 35 | 0.02 |
| Oral Processing | | | | |
| Boli/d | 794 | 690 | 86 | 0.28 |
| Grazing mastication/d | 5,341 | 9,660 | 795 | <0.001 |
| FB mastication/d | 5,969 | | 343 | <0.001 |
| Prehension/d | 18,666 | 30,260 | 2020 | <0.001 |
| Rumination chewing/d | 33,095 | 20,268 | 2432 | <0.001 |
| Total jaw movements/d | 60,316 | 63,897 | 5090 | <0.001 |
| Daily bout data | | | | |
| Grazing bouts | 14.1 | 13.8 | 1.49 | 0.89 |
| Ruminating bouts | 16.6 | 15.5 | 2.07 | 0.66 |
| Idle bouts | 36.9 | 30.6 | 2.83 | 0.104 |
| Supplement bouts | 3.12 | 0 | 0.07 | <0.001 |
| Grazing (min/bout) | 30 | 44 | 3.1 | <0.001 |
| Rumination (min/bout) | 34 | 32 | 3.1 | 0.50 |
| FB (min/bout) | 42 | - | 2.1 | - |
| Idle (min/bout) | 8 | 10 | 1.2 | 0.42 |
| Grazing (mastication/bout) | 371 | 765 | 100.5 | <0.001 |
| Rumination (chewing/bout) | 2,117 | 1,748 | 279 | 0.22 |
| FB (mastication/bout) | 1,813 | - | 200 | - |
| Grazing prehension/bout | 1,235 | 2,305 | 213 | <0.001 |
| Boli/ rumination bout | 50 | 52 | 4.4 | 0.79 |

[1] SE: standard error of estimated marginal means.

methods used to estimate rumen turnover and degradation of NDF are within the range of previous reports (Miller, 2008).

Ruminal DM pools were similar between treatments, and total digesta DM weight declined 38% during the fasting period, which is consistent with previous reports for lactating dairy cows 9-10-h post-prandial (Chilibroste et al., 2000). The DM disappearance of perennial

ryegrass from Dacron bags inserted in the rumen of cows fed FBH declined 24% at 20 h of incubation, compared with those incubated in cows fed HO (56 versus 80% DM disappearance). The DM disappearance of perennial ryegrass observed in the present study is consistent with the report of Barrell et al. (2000), in which 80% of masticated perennial ryegrass was degraded *in sacco* following 20 h of incubation. The decline of herbage DM disappearance *in sacco* in the FBH treatment further supports our conclusion that moderate amounts of FB can reduce the microbial degradation of perennial ryegrass.

In support of our hypothesis, supplementing perennial ryegrass with FB reduced the comminution of large particles in the rumen. While the pool of large particles in the rumen was not different between treatments at the first bailing session at midnight, the pool of large particles following fasting was 27% greater in cows fed FBH than those fed HO. Particle comminution determines the rate of degradation and ruminal passage as smaller particles have a greater surface area available for microbial attachment, and particles < 1.18mm can freely pass the ruminal-reticular orifice (Yang and Beauchemin, 2009; Zebeli et al., 2012a). The greater pool of large particles observed in the rumen of FBH cows was surprising, given FBH cows ruminated longer and grazed less intensively by reducing the number of prehensions per grazing bout by 1,070, compared with those fed HO. The reduced comminution of the large particle pool and increased physical degradation of forage in cows fed FBH suggests the microbial degradation of fibre was less than HO. Pacheco et al. (2020) also found that feeding 45% of DMI as FB increased the proportion of large particles, 40% preprandial, and 27% post-prandial compared with cows fed a harvested herbage diet. The fraction of large particles post-prandial of all cows reported by Pacheco et al. (2020) was greater than observed currently (26.5 vs 37.4 g/100g), reflecting the different time of digesta collection relative to feeding. The increased time spent ruminating may also explain the lack of effect of the FBH treatment on NDF turnover and degradation rate, which would be expected to decline under low ruminal fluid pH conditions.

### 5.5.3 Grazing behaviour

Interestingly, the FBH treatment spent just 7% of their daily activity eating FB; yet, the number of mastications while eating FB was 619 greater than the number of mastications counted during grazing each day. The decline of herbage mastication observed in the FBH treatment may also be explained by the variation of NDF across the sward horizon, which may have reduced tensile strength and mastication needed to ingest herbage. There have not been any

prior experiments reporting the effect of supplementing herbage with FB on oral processing. Pacheco et al. (2020) hypothesised that feeding harvested and chopped FB bulb would increase the particle size of the boli compared with cows which grazed FB crop in situ. However, our results indicate that cows spend more time per kg DM masticating and processing FB bulb before ingestion than while grazing herbage. Cows consume FB when grazed in situ by stabilizing the bulb with their dental pad and scrapping FB pieces from the bulb using their lower incisors. Therefore, the method used to feed FB (e.g., grazing or feeding out harvested bulbs) may alter oral processing, particle size, the rate of ruminal degradation, VFA accumulation, and the risk of cows developing SARA from FB. Further research is needed to identify the impact of either grazing FB *in situ* or feeding harvested and chopped FB bulb to cows on the rate of FB degradation in the rumen and the pH of the ruminal fluid.

The decline of grazing and increase of rumination time observed in cows fed FB was expected. However, the reduced time available for grazing due to the FB meal (82 min/d) did not account for the reduced grazing time (116 min/d) and increased time spent ruminating (+ 86 min/d) compared with the HO diet. Bargo et al. (2003) has previously reported the time spent grazing is expected to decline 12 min/kg of concentrate supplement. In comparison, we observed that the grazing time of cows fed FBH declined 20 min/kg DM of FB consumed. Furthermore, the shorter bout duration of each grazing and reduced grazing intensity (mastication/grazing bout) indicate that cows fed FB were satiated earlier in the meal than those fed HO (Gregorini et al., 2009b). Cows fed the FBH diet spent more time ruminating but did not ruminate with greater regurgitation frequency (i.e., the number of boli was not influenced by diet). However, chewing intensity while ruminating increased in the FBH treatment, indicating a greater amount of energy was expended on processing the FBH diet. An alternative explanation may be due to the increased incidence of pseudo-rumination due to delayed return of fibrous material to the reticulum and the inability to form a solid boli, which may also explain why the FBH cows were idle for 90 min longer each day than the HO treatment (Deswysen and Ehrlein, 1981). However, the current results do not support this conclusion as we did not detect any diet by time interaction for solid and liquid fractions of rumen digesta, which would indicate cows fed FBH may have been unable to form a solid boli.

Further research of the particle fractions in the regurgitated boli may explain the observed increased time and chewing intensity while ruminating by cows fed FBH. We propose that the greater rumination time observed in the FBH treatment plus, the greater

chewing per bolus may improve particle comminution and outflow from the rumen. While FBH reduced the comminution of large particles over the fasting period, the comminution of medium and small particle pools was similar to cows fed HO. Therefore, the increased time spent ruminating, plus the extra chewing per bolus may have helped to maintain rumen function – due to increased saliva flow to the rumen - and milk production of the FBH treatment.

Muscle contractions of the rumen act to either mix (primary contractions) or regurgitate (secondary contractions) digesta; however, rumen motility and rumination are often reduced during acute or lactic ruminal acidosis (Huber, 1976; DeVries et al., 2009). The comminution of particles occurs largely through rumination; although masticating while eating, rumen motility and salivation are important processes that aid microbial adhesion and digestion of feed particles (Maekawa et al., 2002; DeVries et al., 2009). It is expected that the increased mastication caused by the FBH diet would have increased salivation (Beauchemin, 2018). Therefore, the increased rumination of cows fed FBH may have also been a regulatory response to low rumen pH. Williams et al. (2006) also reported that cows experiencing mild SARA spent more time ruminating and masticating when grazing dairy cows were supplemented with cereal grains. Furthermore, the time spent ruminating has been positively related ($R^2$ = 0.98) with the time that pH is below 5.8 (DeVries et al., 2009). Therefore, it is possible that supplemented grazing dairy cows increase rumination time, plus chewing and mastication intensity to aid the decline of microbial activity of the rumen and to increase ruminal fluid pH.

## 5.6 Conclusions

The results indicate that supplementing spring herbage with moderate amounts of FB bulb (~ 40% of DMI) during early lactation reduces the pH of ruminal fluid and ruminal degradation of a perennial ryegrass herbage. The increased time spent ruminating, chewing intensity while ruminating plus ingestive mastication observed in the FBH treatment provides further evidence that cows respond to low ruminal pH by increasing oral processing. The increase of oral processing in the FBH treatment may also increase salivation of neutralizing buffers, although further investigation is needed to confirm this observation. We conclude that supplementing spring herbage with harvested FB bulb reduces grazing time, causes certain individuals to develop SARA and does not benefit early-lactation milk production. Dairy producers should consider alternative feed sources if they are available.

# Chapter 6

# *In vitro* fermentation of fodder beet root increases the cumulative gas production of methane and carbon dioxide.

Published in the Journal of Livestock Science (July 2020)

## 6.1 Abstract

The relationship between the proportion of fodder beet root (FB: *Beta vulgaris* subsp*. vulgaris L.*) in a perennial ryegrass (*Lolium perenne*) diet and gas production was investigated *in vitro*. Cumulative gas production and fermentation products were measured in calibrated glass syringes using a factorial arrangement (4 x 6), of dry matter proportion of FB (0, 15, 35 and 50 % DM: 0FB, 15FB, 35FB and 50FB, respectively), and incubation time (0, 2, 4, 8, 12 and 24 h). Concentrations of methane ($CH_4$) and carbon dioxide ($CO_2$) produced in fermentation and bicarbonate buffering of volatile fatty acid were also calculated. The proportion of FB root linearly increased the volume of gas (mL) produced per g dry matter (DM) and g organic matter (OM). The accumulation of total gas increased 14.8% for 50FB compared with 0FB following 24-h of incubation ($P < 0.001$). The concentration of propionic and butyric acids also increased relative to the proportion of FB ($P < 0.001$). Stoichiometric calculations indicate that FB increased $CO_2$ released from both buffering and fermentation processes but reduced methane fractions in gas (17.6, 17.4, 17.0 and 16.8 ± 0.4 % for 0FB, 15FB, 35FB, and 50FB, respectively, $P< 0.001$). However, the greater OM content of FB increased the total volume of both $CO_2$ and $CH_4$ produced. The increase of propionate concentrations in response to the amount of FB that is fermented limits the H which is available for methanogenesis. However, the greater OM content of FB compared with ryegrass increased total $CO_2$ and $CH_4$ produced from fermentation, resulting in greater emission of gasses per kg DM of the substrate. Further investigation *in vivo* is required to determine the potential effects of microbial protein synthesis on gas production and further verify these results.

## 6.2 Introduction

The rapid incorporation of FB into pastoral grazing systems of New Zealand has preceded research of its impact on ruminant production. Supplementing a ryegrass-based diet with FB

may increase butyrate synthesis and the percentage of fat in milk, but does not increase milk production (Chapter 3 and 4). However, fermentation thermodynamics and gas production rate from a diet containing a mixture of perennial ryegrass and FB have not been evaluated.

Methane and $CO_2$ are potent greenhouse gases produced from microbial fermentation of carbohydrates and amino acids (Johnson and Johnson, 1995). The 2017 report of the Ministry for the Environment estimated that agriculture was responsible for 46% of New Zealand's GHG emissions. Given social and environmental pressure, development of feeds and feeding regimes which limit GHG emissions is crucial to the progression of agriculture. Additionally, enteric $CH_4$ production is an inefficient conversion of energy, representing approximately 2-12% of GE which is un-utilised by the ruminant for maintenance, growth and lactation (Johnson and Johnson, 1995; Howden and Reyenga, 1999). Formation of $CH_4$ is dependent on the availability of hydrogen (H) in the rumen, which is a product of animal intake and the chemical composition of the diet (Johnson and Johnson, 1995). While microbial production of $CH_4$ is the most prolific H sink, propionate formation is also a pathway for H use. Alternatively, the formation of acetate and butyrate result in the net release of H and increase enteric $CH_4$ emissions from livestock (Johnson and Johnson, 1995). Analysis of the volatile fatty acid (VFA) profiles of cows fed FB suggest the concentration of butyrate and propionate increase when FB is used to supplement a ration-based diet (Eriksson et al., 2004). While Jonker et al. (2016) reported daily $CH_4$ emissions (g $CH_4$/kg DMI) declined 28% in non-lactating cows fed ~50% FB and 50% pasture silage, there was no effect on $CH_4$ produced per kg DM consumed when FB represented only 20% of daily dry matter intake (DMI). Furthermore, the kinetics of the end products of fermentation and the net effects on $CH_4$ yield from a FB-ryegrass diet suitable to meet the nutrient demands of lactation, require further definition.

The root of FB is comprised of ~72 % soluble carbohydrate, but is a poor source of crude protein (CP: 7.9%), acid detergent fibre (ADF; 6.7%), neutral detergent fibre (NDF; 11.7%), and can impair animal welfare by causing ruminal acidosis (Dalley et al., 2017). Consequently, it is recommended that FB not exceed 30% of daily DMI when used to supplement herbage during lactation (Dalley et al., 2019; Waghorn et al., 2019). While FB is readily fermentable (Waghorn et al., 2018), the marginal milk response to supplementing medium quality herbage or a mixed ration diet with FB appears to be less than alternative supplements, such as starch dense cereal grains (Roberts, 1987; Fisher et al., 1994; Ferris et al., 2003), or herbages (Chapter 3). The low milk response to FB may be a consequence of

reduced gross energy content < 16 MJ GE/kg DM, or altered pathways of ruminal fermentation (Clark et al., 1987; Waghorn et al., 2018).

The objective of this study was to define how the proportion of DM supplementation of a ryegrass-based herbage diet with FB root would alter the formation of fermentation end-products and gas production *in vitro*. We hypothesised that the proportion of FB would reduce pH and increase the formation of butyrate and propionate and the total rate of gas production.

## *6.3* Methods

### 6.3.1 Experimental design

Fermentation characteristics and cumulative gas production were assessed *in vitro*, in 100 ml glass syringes, fitted with a lubricated piston using the technique outlined by Menke et al. (1979). In a 4 x 6 factorial design, FB was incorporated with a perennial ryegrass diet (PRG) at rates of either 0, 15, 35 or 50% DM (0FB, 15FB, 35FB, and 50FB) and incubations were stopped at either 0, 2, 4, 8, 12 or 24 h to measure cumulative gas production and the products of fermentation. Each time by FB level was duplicated, and incubations were carried out on two separate occasions (runs) resulting in four observations per diet-by-time combination. Within each run, three syringes free of substrate were also included as blank incubations.

### 6.3.2 Sample preparation

In the autumn (April) of 2018, samples of perennial ryegrass were collected 3-4 weeks following defoliation to ~ 1550 kg DM/ha, and the application of urea (100 kg/ha). Herbage was an established perennial ryegrass (85.6 ± 7.04 % DM of sward) and white clover (W.C: *Trifolium repens*; 8.8 ± 5.0 % DM of sward) sward from the Lincoln University Research Dairy Farm (-43.64, 172.45), harvested above ground, frozen (-20℃), freeze-dried and ground by a centrifugal mill (ZM200 Retsch GmbH; Haan, Germany) to pass a 1mm sieve. Fodder beet, c.v. Jamon, was sown in late spring 2016 and two quadrats 1 m wide and 2 m long were harvested in July 2017. Fodder beet was weighed, and roots were defoliated then horizontally quartered. One quarter was homogenised in a food processor and sub-sampled to determine DM (oven-dried at 100 ℃ for 48 h). The remaining material from each root was composited and frozen at -20 ℃ until freeze-dried and ground through a fine sieve (1 mm) before chemical analysis and incubation.

### 6.3.3 Incubation

Two litres of rumen fluid were collected at approximately 0800 h before morning feeding, from two non-lactating Jersey x Friesian cows (1 L /cow) with the approval of the Lincoln University Animal Ethics Committee (AEC 2018-24). Prior to collection, cows grazed a PRG and WC sward (~10 kg DM/cow/d). Digesta was collected through a ruminal cannula by grabbing digesta in random locations and squeezing the digesta to collect liquid in the thermos, following the method outlined in Menke et al. (1979). Upon collection, ruminal fluid was stored in a pre-heated (39.5°C) thermos which was purged with $CO_2$ and transported to the lab (~ 10 minutes post-collection) where it was immediately strained through Chux cheesecloth and added to the buffer medium outlined in Menke et al. (1979) at a 1:2 ratio of ruminal fluid to buffer. The mixture of rumen fluid and buffer medium was kept at 39.5°C in a water bath and continuously purged with $CO_2$ to maintain anaerobic conditions while syringes were loaded.

Syringes were incubated in a shaking (60 rpm) oven rack at 39.5ºC with 200 mg of the substrate and 30 mL of the rumen fluid-buffer solution. Syringes were removed at 0, 2, 4, 8, 12, or 24 h, depending on the allocation. The piston was read as a measure of cumulative gas production (mL) at the end of the incubation. Upon removing the syringes from the oven rack, fluid was immediately measured for pH using a benchtop meter (Orion 2-star, Thermo Scientific, Beverly, USA) and placed on ice to stop fermentation. Samples of the buffer and rumen fluid mixture were collected and stored in 2-mL Eppendorf tubes for assessing VFA content and acidified (10 µL of 99% H2SO4) to determine the concentration of ammonia ($NH_3$). The gas produced (ml) was corrected for blank values and expressed as ml/g of DM and organic matter (OM) of the substrate.

### 6.3.4 Chemical analysis and calculations

Samples of ground FB root and herbage were analysed for nitrogen (N): Elementar (Variomax CN Analyser, Elementar Analysensysteme, Germany), acid detergent fibre (ADF) and neutral detergent fibre (NDF) following the methods of (Van Soest et al., 1991a), ash and WSC using the procedure outlined in Pollock and Jones (1979). Volatile fatty acid concentrations were detected using gas chromatography (model GC-2010, Shimadzu, Kyoto, Japan), 20 µl of 2-methylvaleric acid internal standard and 40 µl metaphosphoric acid were added to 100 µl of centrifuged sample vortexed, diluted with 50/50 acetone/water and filtered using a 0.22 µm

filter prior to GC injection. Briefly, 1 µL was injected using an autosampler (AOC-20i) at a split ratio of 1:3 at the injection port (240°C). The VFA was separated on a bore capillary column (SGE BP21 30 m x 530 µm x 1.0 µm) with a flow of 5.23 mL/min of Helium. The initial oven temperature was 105°C held for 4 min and then increased by 15°C/min to 230°C, where it was held for 5 min. The flame ionization detector was maintained at 240°C. Ammonia concentrations were determined by an enzymatic UV method using a Randox $NH_3$ kit by the Randox Rx Daytona analyser ($NH_3$ clinical assay kit: AM 3979, Randox Laboratories, Ltd, United Kingdom).

Fractions of $CH_4$ ($CH_{4ferm}$) and $CO_2$ ($CO_{2ferm}$) produced from fermentation and buffering ($CO_{2buff}$) were calculated using stoichiometry of VFA products as outlined by Wolin (1960) and developed by others (Blümmel et al., 1997; Blümmel et al., 1999).

Fermentation products; $CO_2$ and $CH_4$ were calculated as:

$$CO_{2ferm}\ \text{mmol} = (a/2 + p/4 + 1.5b) \quad (6.1)$$

$$CH_{4ferm}\ \text{mmol} = (2b + a - CO_{2ferm}) \quad (6.2)$$

Where *a*, *p* and *b* are proportions of the sum of acetate, butyrate and propionate of each syringe and $CO_{2ferm}$ is derived from equation 5.1. Carbon dioxide produced from buffering ($CO_{2buf}$) of VFA was calculated assuming that 1 mmol of VFA will produce 1 mmol of $CO_{2buff}$ including iso-butyrate, formed by fermentation of amino acids.

$$CO_{2buff}\ \text{mmol} = (a + b + p + \text{iso-butyrate}) \quad (6.3)$$

The percentage of $CO_{2ferm}$, $CH_4$, and $CO_{2buff}$ were then calculated per unit of OM and DM of the substrate.

### 6.3.5 Statistical analysis

Total gas production (ml/g DM and ml/g OM) $CO_2$, $CH_4$, concentrations of VFA, $NH_3$ and pH were statistically analysed as a factorial design using a mixed-effects ANOVA using the 'nlme' package (Pinheiro et al., 2018) in R (R Core Team, 2018, v. 3.4.4.) where the syringe was the experimental unit, FB inclusion, incubation time. The interaction of diet by incubation time was the fixed effect, and random effects were time nested within the run. The model of Ørskov and McDonald (1979) did not fit the current data set as fermentation had not plateaued at 24-

h following the start of incubation and limited rack size and measurement of fermentation end-products at each time-point prevented analysis of fermentation at 48 h incubation. However, complete *in vivo* ruminal clearance of non-structural components and NDF of the substrate within 24-h of incubation have been previously reported (Menke and Steingass, 1988; Taweel et al., 2006; Williams et al., 2006). Upon significance of the ANOVA, orthogonal polynomial contrasts were used to test the significance of linear, quadratic and cubic components across the level of FB allocated using the 'emmeans' package (Lenth, 2018). Differences were declared significant if $P \leq 0.05$ and tendencies were $0.05 < P \leq 0.1$.

## 6.4 Results

### 6.4.1 Gas production

Compared with ryegrass-herbage, FB contained 65.3% less N and CP, 68% less ADF, 66% less NDF and the proportion of WSC in FB root was 78% greater compared with PRG herbage (Table 6.1). Significant effects ($P < 0.05$) of incubation time were detected for all variables reflecting the accumulation of fermentation products over the incubation period. Significant ($P < 0.01$) time by FB inclusion interactions were detected for gas produced per g DM and g of OM, although differences were not detected until after 4 h of incubation (Table 6.2). Linear and quadratic effects of the diet were detected at 8 h of incubation as the 15FB diet produced 10 ml gas/g DM less than 0FB (Figure 6.1, Table 6.2). Linear effects of the diet were also detected at 12 and 24 h of incubation. A cubic tendency ($P = 0.06$) for 30FB to produce more gas than 50FB at the 12 h interval was also observed (Table 6.2). Linear effects of FB inclusion by incubation time were also detected for gas produced per g of OM, as gas volume increased with the level of FB from hour eight onwards (Table 6.2, Figure 6.1).

### 6.4.2 Volatile fatty acids and ammonia

Time × treatment interactions were significant for concentrations of $NH_3$, butyrate and branched-chain VFA (Table 6.2). The concentrations of $NH_3$ declined with increasing FB inclusion (Table 6.2). Significant time × treatment interactions were detected ($P < 0.001$) between eight and 24 h incubation (Figure 6.2). Concentrations of butyric acid increased linearly between 12 and 24 h of incubation, while branched-chain VFA: iso-butyrate and iso-valerate, declined in response to the increased percentage of FB (Figure 6.2). While the incubation time was significant, treatment by time interactions for pH, acetate, propionate, hexanoate, valerate, A:P or total VFA concentration were not significant. Therefore, only

treatment effects are discussed. Differences in acetate, valerate, hexanoate, and total VFA response to FB inclusion were not significant (Table 6.3). However, the concentration of propionate increased with FB proportion in the diet ($P < 0.001$) and caused the A:P ratio to decline with increasing FB inclusion (Table 6.3). The dietary proportion of FB also reduced pH linearly, but differences were within a 0.1 range of pH ($P< 0.001$; Table 6.3).

### 6.4.3 Stoichiometric calculations

While time by treatment interactions of $CH_4$, $CO_{2buff}$ and $CO_{2ferm}$ fractions were not significant, the fractions of $CO_2$ and $CH_4$ increased ($P < 0.01$) as incubation progressed, peaking at 12 h of incubation and remaining constant until 24 h (only data from hour 24 shown; Table 5.4). The proportion of $CH_4$ declined linearly, while fractions of $CO_{2buff}$ increased linearly relative to the amount of FB incubated (Table 4). Time $\times$ treatment interactions for the production of $CH_4$, $CO_{2ferm}$ and $CO_{2buff}$ (ml/g OM) were also evident, and the production of all gasses increased linearly with FB (Figure 5.3). While FB increased the volume of $CH_4$ per g DM (Table 5.5), the yield of $CH_4$/g OM declined with the increasing proportion of FB. While significant differences in $CH_4$ yield (ml/g OM) at 8 and 12 h of incubation were observed, there were no significant differences in the diet at 24 h of incubation (Figure 5.3).

Table 6.1.The chemical composition[1] and dry matter[2] content of fodder beet root (FB) and a perennial ryegrass-based herbage used in diets containing ryegrass and increasing proportion of FB (0, 15, 35 and 50% FB; 0FB, 15FB, 35FB and 50FB, respectively)

| Chemical Component[1] | FB root | Herbage (0FB) | Diet | | |
|---|---|---|---|---|---|
| | | | 15FB | 35FB | 50FB |
| Dry matter % | 13.7 ± 0.71 | 17.6 ± 1.86 | - | - | - |
| NDF % | 11.8 | 34.7 | 31.3 | 26.7 | 23.2 |
| ADF % | 6.56 | 20.6 | 18.5 | 15.7 | 13.6 |
| Nitrogen % | 1.35 | 3.9 | 3.5 | 3.0 | 2.6 |
| CP % | 8.4 | 24.3 | 21.9 | 18.7 | 16.3 |
| WSC % | 67.5 | 14.9 | 22.8 | 33.3 | 41.2 |
| OM % | 92.9 | 88.6 | 89.2 | 90.1 | 90.7 |
| Ash | 7.1 | 11.4 | 10.8 | 9.9 | 9.3 |

[1] NDF: neutral detergent fibre, ADF: acid detergent fibre, CP: crude protein, WSC: water-soluble carbohydrate and OM: organic matter. [2] DM, (mean ± standard error)

Table 6.2. Cumulative gas production (ml) per g of dry matter (DM) and organic matter (OM) of the substrate and fermentation-end products during fermentation. Diets contained perennial ryegrass with increasing percentage of fodder beet root (FB: 0, 15, 35 and 50% FB; 0FB, 15FB, 35FB and 50FB, respectively), all variables presented, displayed significant treatment by time interactions.

| | | Diet | | | | | *P*-Value[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Time** | 0FB | 15FB | 35FB | 50FB | **SEM[1]** | L | Q | C |
| Gas ml/g DM | 2 | 11.9[a] | 17.4[a] | 18.6[a] | 19.4[a] | 7.62 | 0.56 | 0.77 | 0.91 |
| | 4 | 26.9[a] | 41.7[a] | 48.0[a] | 34.6[a] | 7.62 | 0.40 | 0.08 | 0.74 |
| | 8 | 98.4[a] | 88.6[a] | 115[ab] | 140[b] | 7.62 | *** | * | 0.28 |
| | 12 | 123[a] | 137[a] | 172[b] | 163[b] | 7.62 | *** | 0.14 | 0.06 |
| | 24 | 193[a] | 192[a] | 205[a] | 226[b] | 7.62 | ** | 0.16 | 0.88 |
| Gas ml/g OM | 2 | 13.3[a] | 19.5[a] | 20.7[a] | 21.3[a] | 8.49 | 0.92 | 0.99 | 1.00 |
| | 4 | 30.3[a] | 46.7[a] | 53.3[a] | 38.1[a] | 8.31 | 0.82 | 0.21 | 0.99 |
| | 8 | 111[a] | 99.2[a] | 128[ab] | 154[b] | 8.31 | *** | 0.10 | 0.64 |
| | 12 | 138[a] | 153[a] | 191[b] | 179[b] | 8.31 | *** | 0.33 | 0.20 |
| | 24 | 217[a] | 215[a] | 228[ab] | 250[b] | 8.31 | * | 0.45 | 1.00 |
| Ammonia (mmol/L) | 2 | 9.45[a] | 8.83[a] | 8.60[a] | 9.80[a] | 0.552 | 0.97 | 0.19 | 0.95 |
| | 4 | 8.85[a] | 8.29[a] | 7.73[a] | 7.79[a] | 0.552 | 0.24 | 0.89 | 0.99 |
| | 8 | 8.93[a] | 7.97[a] | 6.77[b] | 5.82[b] | 0.552 | *** | 1.00 | 0.99 |
| | 12 | 9.05[a] | 7.76[ab] | 6.59[b] | 6.19[b] | 0.552 | *** | 0.74 | 0.99 |
| | 24 | 12.4[a] | 11.5[a] | 9.70[b] | 8.00[bc] | 0.552 | *** | 0.81 | 0.97 |
| Butyrate (mmol/L) | 2 | 2.52[a] | 2.59[a] | 2.69[a] | 2.64[a] | 0.771 | 0.87 | 0.97 | 0.99 |
| | 4 | 2.55[a] | 2.66[a] | 2.73[a] | 2.55[a] | 0.771 | 1.00 | 0.73 | 0.99 |
| | 8 | 3.17[a] | 3.27[a] | 3.03[a] | 3.30[a] | 0.771 | 0.99 | 0.92 | 0.99 |
| | 12 | 3.66[a] | 3.81[a] | 4.38[b] | 4.24[b] | 0.771 | ** | 0.92 | 0.53 |
| | 24 | 4.06[a] | 4.33[a] | 4.63[b] | 5.04[a] | 0.771 | *** | 0.95 | 1.00 |
| Iso-butyrate (mmol/L) | 2 | 0.26[a] | 0.27[a] | 0.26[a] | 0.25[a] | 0.054 | 0.98 | 0.85 | 0.90 |
| | 4 | 0.27[a] | 0.27[a] | 0.27[a] | 0.25[a] | 0.054 | 0.65 | 0.89 | 0.90 |
| | 8 | 0.35[a] | 0.34[ab] | 0.26[b] | 0.25[b] | 0.054 | *** | 0.98 | 0.26 |
| | 12 | 0.39[a] | 0.35[a] | 0.29[b] | 0.25[b] | 0.054 | *** | 0.98 | 0.95 |
| | 24 | 0.54[a] | 0.52[a] | 0.42[b] | 0.41[b] | 0.054 | *** | 0.97 | 0.06 |
| Iso-valerate (mmol/L) | 2 | 0.43[a] | 0.46[a] | 0.40[a] | 0.43[a] | 0.118 | 0.97 | 1.00 | 0.41 |
| | 4 | 0.43[a] | 0.44[a] | 0.42[a] | 0.39[a] | 0.118 | 0.43 | 0.80 | 1.00 |
| | 8 | 0.52[a] | 0.45[a] | 0.36[b] | 0.31[b] | 0.118 | *** | 0.99 | 0.79 |
| | 12 | 0.55[a] | 0.47[a] | 0.38[b] | 0.33[b] | 0.118 | *** | 0.93 | 0.94 |
| | 24 | 0.86[a] | 0.80[a] | 0.64[v] | 0.58[v] | 0.118 | *** | 1.00 | 0.23 |

[1]SEM: standard error of the mean, [a-b] Different superscripts within rows are significantly different $P<0.05$. [2]Differences are displayed as linear (L), quadratic (Q) or cubic (C) effects.* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

## 6.5 Discussion

### 6.5.1 Cumulative gas production and rate

The increased gas production per g DM in response to the amount of FB in the diet suggests both the rate and the extent of FB degradation was greater than ryegrass, consistent with previous analyses (Waghorn et al., 2018). The gas produced following 24 h of incubation ranged from 193-226 mL/g DM, which is slightly greater than the batch-culture production of 178-189 mL/g DM reported previously (Purcell et al., 2014). Differences are likely due to a combination of different methodologies, donor animal, donor animal diet and the chemical composition of the substrate (Getachew et al., 2002). The concentration of WSC and OM of the substrate is known to increase gas production from fermentation *in vivo* and *in vitro* (Blümmel and Ørskov, 1993; Johnson and Johnson, 1995) likely account for the positive relationship between gas production and the percentage of FB in the substrate. While FB also increased gas production per g of OM, differences were not significant at 24 h incubation, providing further evidence that the high OM content of FB increased cumulative gas production.

The addition of FB in the diet increased the yield of gasses from 8 h of incubation. Approximately 40% of the biomass of FB is comprised of sucrose (Giaquinta, 1979; Clark et al., 1987), which is degraded very quickly in the rumen (300% per hr) compared with starch (10-15% per hr) or pasture (DM ~7.6 % per hr) (Sniffen et al., 1992). Sucrose is stored in thin-walled parenchyma cells in FB roots, which are completely digested within 8 h of incubation (Jung and Engels, 2001). The degradation of parenchyma cells throughout the first 8 h of incubation may explain the sudden and rapid accumulation of gasses observed with FB. While others have reported that fermentation of sucrose (including its' derivatives; glucose and fructose) occur at a faster rate than lactose, arabinose or mannitol, the type of sugar incubated does not appear to alter total gas production over 24-h of fermentation (Ahmed et al., 2013; Purcell et al., 2014). Therefore, the increase of total gas production with the inclusion of FB root reflect altered carbohydrate content of diets.

Digestion of the epidermal, mesophyll and fibre cells of pasture are slow, owing to the presence of primary and secondary plant cell walls (Chesson et al., 1986). The structural primary cell wall contains polysaccharides: cellulose, lignin, hemicellulose, and pectin, comprising up to 80% of plant DM, depending on the phonological state (Wilson, 1994). Although Jung and Engels (2001) have shown complete degradation of primary cell wall after 12 h incubation, the linear shape and absence of a plateau of gas production observed in Figure 6.1, may imply incomplete fermentation of perennial ryegrass at 24 h, due to the greater content of structural carbohydrate.

Table 6.3 The effect of increasing the percentage of fodder beet root (FB: 0, 15, 35 and 50% FB; 0FB, 15FB, 35FB and 50FB, respectively) in a perennial ryegrass-based diet on pH and the concentrations of fermentation products.

| **Variable** | **Diet** | | | | **SEM[1]** | ***P*-Value[2]** | | |
|---|---|---|---|---|---|---|---|---|
| | 0FB | 15FB | 35FB | 50FB | | L | Q | C |
| pH | 6.96[a] | 6.95[a] | 6.87[b] | 6.86[b] | 0.021 | *** | 1.00 | 0.08 |
| TVFA[2] (mmol/L) | 42.7[a] | 43.9[a] | 44.3[a] | 44.8[a] | 5.58 | 0.14 | 0.97 | 0.99 |
| Acetate (mmol/L) | 28.7[a] | 29.3[a] | 29.4[a] | 29.7[a] | 2.78 | 0.36 | 0.99 | 0.97 |
| Propionate (mmol/L) | 9.1[a] | 9.6[a] | 10.0[a] | 10.2[ab] | 1.65 | ** | 0.90 | 1.00 |
| Valerate (mmol/L) | 0.47[a] | 0.48[a] | 0.46[a] | 0.46[a] | 0.127 | 0.51 | 0.97 | 0.91 |
| Hexanoate (mmol/L) | 0.23[a] | 0.23[a] | 0.23[a] | 0.22[a] | 0.123 | 0.99 | 0.98 | 1.00 |
| A/P[3] | 3.33[a] | 3.24[a] | 3.16[ab] | 3.15[b] | 0.283 | * | 0.80 | 0.99 |

[a-b] Different superscripts within rows are significantly different $P<0.05$. [2]Significant differences are displayed as linear (L), quadratic (Q) or cubic (C) effects. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[1] SEM: standard error of the mean

[2] Total volatile fatty acids

[3] Acetate: Propionate ratio

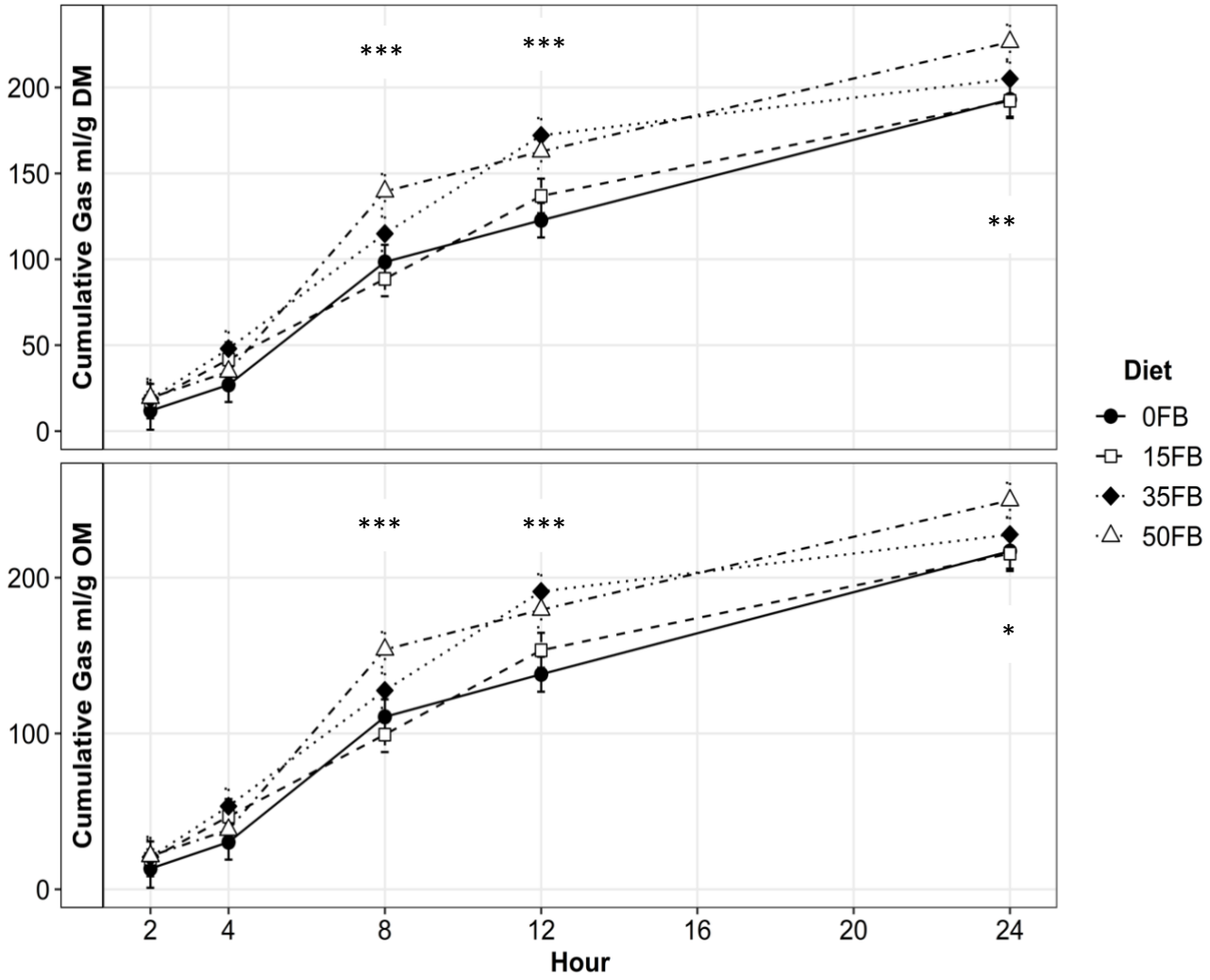


Figure 6.1 Gas production (ml) per gram dry matter (g DM) and organic matter (g OM) of a perennial ryegrass-based diet with increasing proportions (0, 15, 35 and 50%) of fodder beet root (0FB, 15FB, 35FB and 50FB, respectively). Significant diet interactions are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

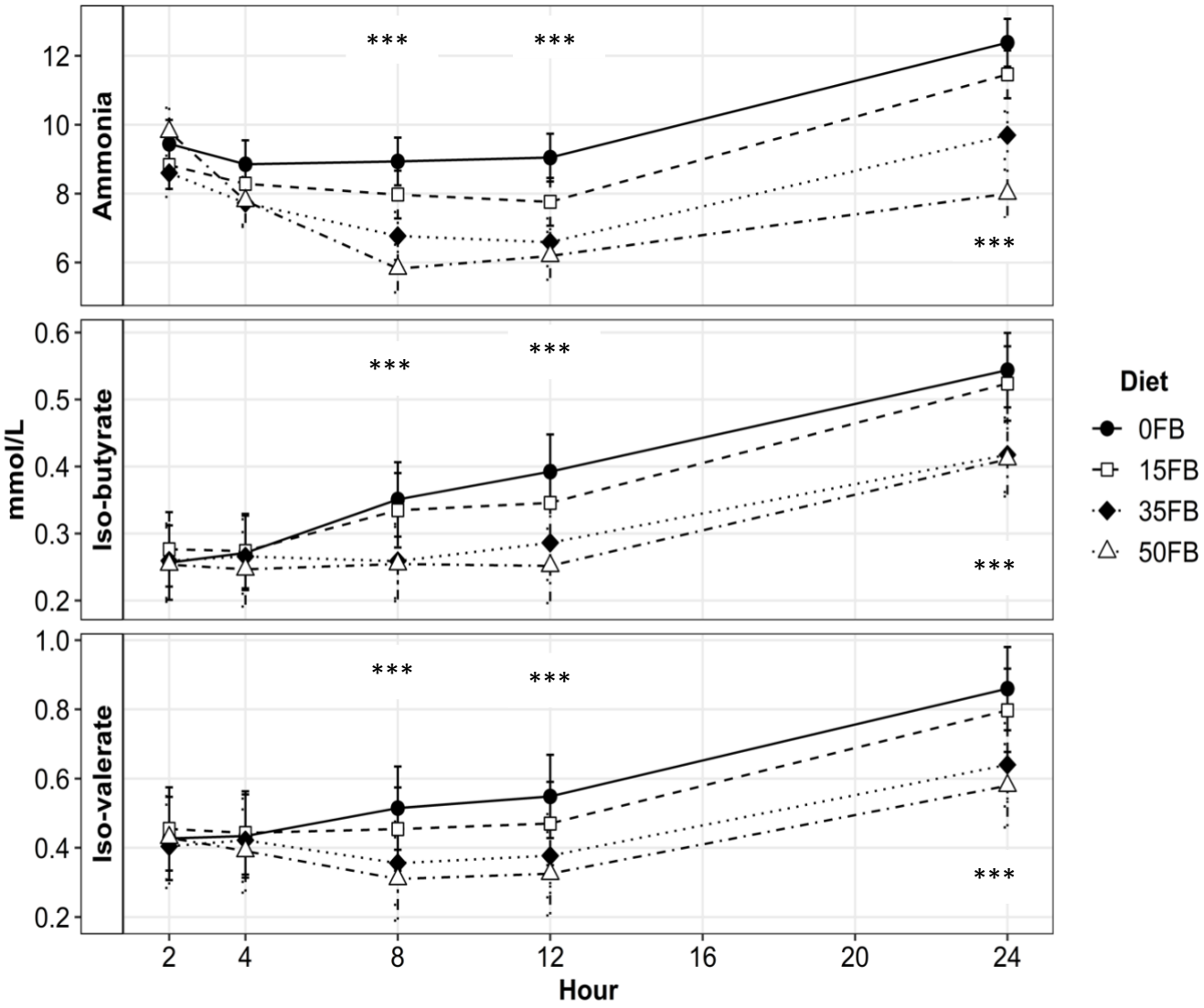


Figure 6.2. Ammonia, iso-butyrate and iso-valerate concentrations (mmol/L) in response to a ryegrass diet with increasing proportion (0, 15, 35 and 50%) of fodder beet root (0FB, 15FB, 35FB and 50FB, respectively). Significant diet interactions are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

### 6.5.2 Gas components

Accumulated gasses include both $CH_4$ and $CO_2$ formed during substrate fermentation ($CO_{2ferm}$) and $CO_2$ released from bicarbonate buffering of VFA ($CO_{2buff}$). Estimated $CO_{2buff}$ accounted for 58% of the gas produced in syringes, similar to the 50% reported by Blümmel and Ørskov (1993). The pH of syringe fluid ranged between 6.96 and 6.86 across treatments which is similar to previous reports and indicates the ability of the buffer to maintain pH was high (Mould et al., 2005). The proportion of $CO_{2buff}$ increased significantly with the amount of FB fermented due to the reduced content of NDF, ADF, and the increased content of WSC (Table 5.3), which also increased $CO_{2ferm}$. The decline of pH with incubation time, and the percentage

of FB was significant statistically but not biologically as time by treatment interactions of total VFA concentration were not significant ($P > 0.05$). However, the increased concentration and yield of $CO_{2buff}$ suggests FB increased the rate of fermentation, VFA formation, and the buffering of pH in syringes (Table 6.3).

Fodder beet root reduced $CH_4$ fractions of gas, despite the increased formation of butyrate. Stoichiometry calculations were originally outlined by Wolin (1960), in which $CH_4$ is a product of acetate and butyrate formation, where butyrate is formed from two moles of acetate and therefore yields twice the amount of $CH_4$ (Equation 6.2). However, the significant increase of propionate concentration reduced the ratio of A:P and reduced the H available for methanogens to reduce $CO_2$ to $CH_4$ (Janssen, 2010). The proportions of VFA produced in fermentation are; acetate > propionate > butyrate (Table 6.3). Thus, H use from propionate exceeded the H produced from butyrate, which explains the inverse relationship observed between the percentage of FB and $CH_4$ yield.

Table 6.4. Fractions of carbon dioxide ($CO_2$) and methane ($CH_4$) produced from diets containing perennial ryegrass and either 0, 15, 35 or 50% fodder beet root (FB: 0FB, 15FB, 35FB and 50FB, respectively).

| Variable | Diet | | | | SEM[1] | *P*-Value[3] | | |
|---|---|---|---|---|---|---|---|---|
| | 0FB | 15FB | 35FB | 50FB | | L | Q | C |
| $CO_{2ferm}$[2] % | 28.0[a] | 28.1[a] | 28.4[b] | 28.4[b] | 0.17 | *** | 1.00 | 0.58 |
| $CH_4$ % | 17.6[a] | 17.4[a] | 17.0[ab] | 16.8[b] | 0.45 | *** | 0.95 | 0.83 |
| $CO_{2buff}$[3] % | 58.0[a] | 58.2[a] | 58.3[a] | 59.0[b] | 0.50 | * | 0.92 | 0.93 |

[a-b] Different superscripts within rows are significantly different $P<0.05$.. [1] SEM: standard error of the mean. [2] $CO_2$ from substrate fermentation. [3] $CO_2$ produced from buffering of organic acids.
[3]Significant differences are displayed as linear (L), quadratic (Q) or cubic (C) effects
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Despite the reduction of $CH_4$ percentage in gas, the productions of $CH_4$/g DM increased relative to the percentage of FB root in the substrate. However, previously Jonker et al. (2016) reported a diet containing equal portions of FB and silage reduced enteric $CH_4$ yield by 28% (g $CH_4$ /kg DMI) in non-lactating cows compared with those fed similar proportions of kale and silage. The discrepancy between *in vitro* and *in vivo* results may reflect the methodology used or the dietary OM content, which increased 2.1% between 0FB and 50FB. The production of $CH_4$ at 24 h incubation increased per g DM but not per g OM (Figure 6.3), indicating the greater OM content of FB may increase total $CH_4$ yield per g of DM when FB exceeds 35% of the diet.

While the fermentation thermodynamics in a closed system as measured *in vitro*, will differ to the thermodynamics in a live animal, further research of enteric methane emissions from cows fed varying amounts of FB, is warranted.

### 6.5.3 Volatile fatty acid profiles

The observed increase of butyrate and propionate in addition to the decline of branched-chain fatty acids agree with data obtained from late lactation dairy cows fed FB and herbage (Pacheco et al., 2020). Fermentation pathways which favour the production of $CH_4$ are regulated by the partial pressure of hydrogen gas ($H_2$) which is a reduced form of $H^+$ (Janssen, 2010). Formation of butyrate indicates that the partial pressure of $H_2$ is low, while the production of propionate suggests greater partial pressure of $H_2$ (Hegarty and Gerdes, 1999). The current results may indicate the faster rate of FB fermentation initially favoured the formation of propionate. Although as fermentation progressed, the increasing concentration of $H_2$ will have caused a shift favouring butyrate formation (Janssen, 2010). However, the partial pressure of $H_2$ is greater in a closed atmosphere (syringe) compared with the rumen (Hegarty and Gerdes, 1999), and significant diet by time interactions was not significant for propionate concentrations, which again highlight the need for further research *in vivo*.

Stoichiometry of gasses produced by fermentation was predicted by assuming that 1 mmol of VFA will produce 1 mmol of $CO_2$, which included iso-butyrate formed from the fermentation of amino acids. Wolin (1960) assumed the gas produced from protein was marginal and was derived completely from carbohydrate fermentation. However, Cone and van Gelder (1999) reported that gas production declined 2.48 mL/g OM per percentage of protein included in the substrate, and from the fermentation of soluble protein in the early stages of incubation. The conversion of ammonia to ammonium binds $H^{+,}$ impairing buffering capacity and reducing $CO_2$ production from fermentation buffers (Cone and van Gelder, 1999). However, Blümmel et al. (1999) found that gas production could be accurately predicted ($R^2$ = 0.95) using the stoichiometry calculations when CP of the substrate was < 40% DM. In comparison, the CP of substrates fermented in the current study was less than 25% DM (Table 6.1), so it is unlikely that the lower gas produced by 0FB was due to increased herbage proportions, or the N content of the diet. The observed decline of pH and increased $CO_{2buff}$ in proportion to FB, suggest the efficacy of the bicarbonate buffer was not affected by the protein content of herbage. However, further evaluation of the microbial

Table 6.5 Volume (mL) of methane ($CH_4$) and carbon dioxide produced by fermentation of substrate ($CO_{2ferm}$) and buffering of volatile fatty acids ($CO_{2buff}$) per g of dry matter (DM) and organic matter (OM) and interaction between incubation time and diet of perennial ryegrass and increasing proportion of fodder beet root (FB) and (0, 15, 35 and 50% FB; 0FB, 15FB, 35FB and 50FB, respectively), all variables presented, displayed significant treatment by time interactions.

| Variable | Time | Diet | | | | | *P*-value[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0FB | 15FB | 35FB | 50FB | SEM[1] | L | Q | C |
| $CH_4$ mL/g DM | 2 | 1.4[a] | 3.3[a] | 3.4[a] | 2.9[a] | 1.85 | 0.82 | 0.74 | 1.00 |
| | 4 | 5.0[a] | 7.6[a] | 8.7[a] | 6.5[a] | 1.85 | 0.73 | 0.21 | 1.00 |
| | 8 | 17.5[a] | 15.5[a] | 20.2[ab] | 24.2[b] | 1.85 | *** | 0.08 | 0.52 |
| | 12 | 21.2[a] | 23.3[ab] | 28.8[b] | 27.3[b] | 1.85 | *** | 0.45 | 0.25 |
| | 24 | 33.4[a] | 33.5[a] | 34.9[a] | 38.0[ab] | 1.85 | 0.07 | 0.45 | 1.00 |
| $CO_{2ferm}$ mL/g DM | 2 | 2.1[a] | 4.9[a] | 53.0[a] | 4.6[a] | 2.86 | 0.81 | 0.81 | 1.00 |
| | 4 | 7.5[a] | 11.7[a] | 13.4[a] | 9.7[a] | 2.86 | 0.79 | 0.21 | 0.98 |
| | 8 | 27.5[a] | 24.7[a] | 32.0[ab] | 38.9[b] | 2.86 | *** | 0.09 | 0.64 |
| | 12 | 34.4[a] | 38.5[a] | 48.9[b] | 46.3[b] | 2.86 | *** | 0.35 | 0.15 |
| | 24 | 54.1[a] | 54.1[a] | 58.1[ab] | 64.4[b] | 2.86 | ** | 0.40 | 1.00 |
| $CO_{2buff}$ mL/g DM | 2 | 4.0[a] | 9.2[a] | 10.1[a] | 8.8[a] | 5.91 | 0.84 | 0.86 | 1.00 |
| | 4 | 14.4[a] | 22.6[a] | 26.2[a] | 18.4[a] | 5.91 | 0.83 | 0.86 | 1.00 |
| | 8 | 54.5[a] | 50.5[a] | 64.7[ab] | 80.1[b] | 5.91 | *** | 0.08 | 0.77 |
| | 12 | 70.5[a] | 79.1[ab] | 100[b] | 94.2[b] | 5.91 | *** | 0.30 | 0.15 |
| | 24 | 111[a] | 111[a] | 120[ab] | 133[b] | 5.91 | ** | 0.35 | 1.00 |
| $CO_{2ferm}$ mL/g OM | 2 | 2.3[a] | 5.5[a] | 5.9[a] | 5.0[a] | 3.18 | 0.82 | 0.80 | 1.00 |
| | 4 | 8.5[a] | 13.1[a] | 14.9[a] | 10.6[a] | 3.18 | 0.83 | 0.21 | 1.00 |
| | 8 | 30.9[a] | 27.7[a] | 35.6[ab] | 42.9[b] | 3.18 | *** | 0.10 | 0.65 |
| | 12 | 38.7[a] | 43.1[a] | 54.2[b] | 51.0[b] | 3.18 | *** | 0.32 | 0.17 |
| | 24 | 60.8[a] | 60.6[a] | 54.5[a] | 71.0[b] | 3.18 | ** | 0.44 | 1.00 |
| $CH_4$ ml/g OM | 2 | 1.5[a] | 3.7[a] | 3.8[a] | 3.2[a] | 2.07 | 0.84 | 0.74 | 1.00 |
| | 4 | 5.6[a] | 8.5[a] | 9.7[a] | 7.2[a] | 2.07 | 0.77 | 0.20 | 1.00 |
| | 8 | 19.6[a] | 17.3[a] | 22.4[ab] | 26.7[b] | 2.07 | *** | 0.90 | 0.53 |
| | 12 | 23.8[a] | 26.1[ab] | 31.9[b] | 30.1[b] | 2.07 | ** | 0.42 | 0.27 |
| | 24 | 38.2[a] | 37.5[a] | 38.8[a] | 41.9[a] | 2.07 | 0.18 | 0.50 | 1.00 |
| $CO_{2buff}$ mL/g OM | 2 | 4.5[a] | 10.3[a] | 11.2[a] | 9.7[a] | 6.59 | 0.85 | 0.86 | 1.00 |
| | 4 | 16.2[a] | 25.3[a] | 29.0[a] | 20.3[a] | 6.59 | 0.87 | 0.23 | 1.00 |
| | 8 | 62.4[a] | 56.6[a] | 71.8[ab] | 88.3[b] | 6.59 | *** | 0.09 | 0.77 |
| | 12 | 79.3[a] | 88.6[a] | 111[b] | 103[b] | 6.59 | *** | 0.27 | 0.16 |
| | 24 | 126[a] | 125[a] | 132[ab] | 147[b] | 6.59 | ** | 0.39 | 1.00 |

[1]SEM: Standard error of the mean, [a-b] Different superscripts within rows are significantly different $P<0.05$, [2]Significant differences are displayed as linear (L), quadratic (Q) or cubic (C) effects. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

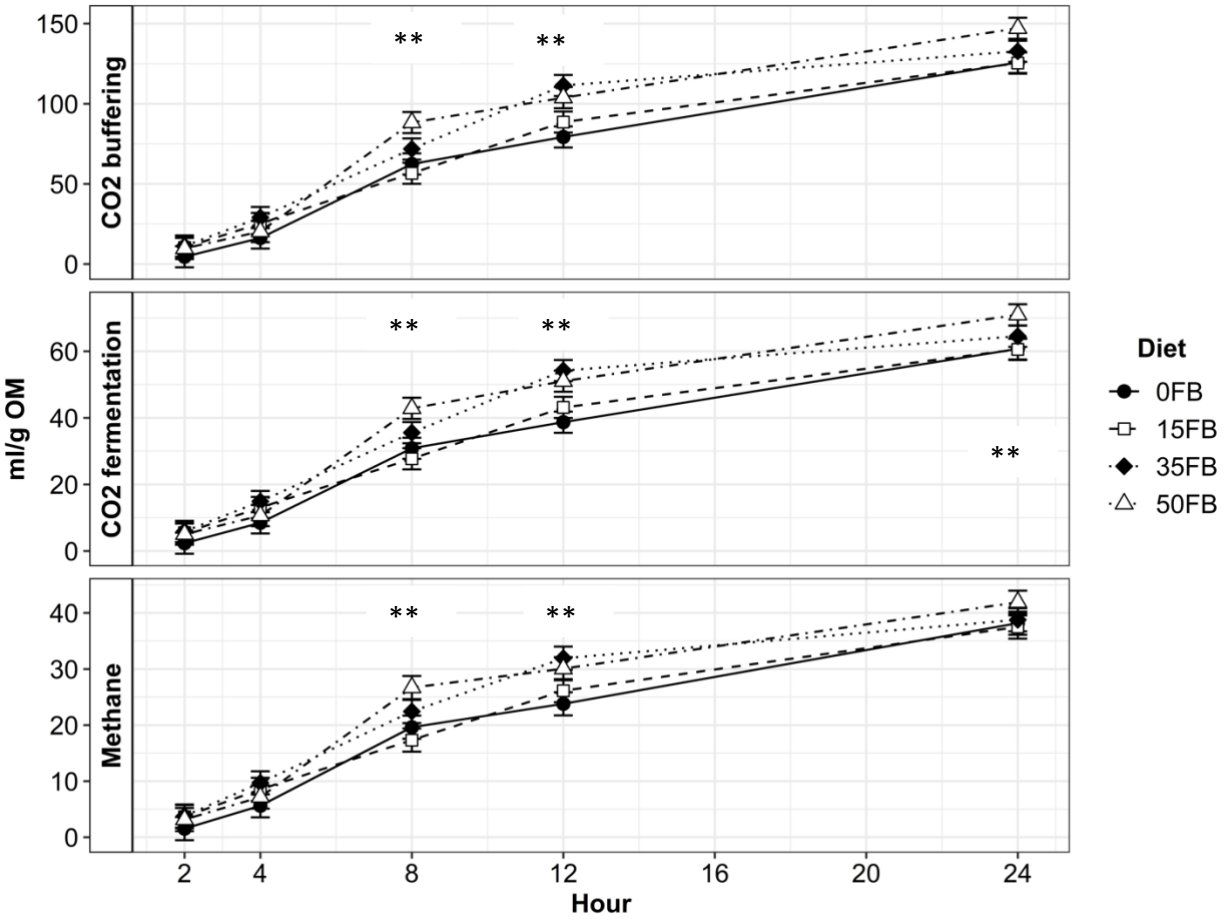


Figure 6.3. Time by diet interactions of methane ($CH_4$) and carbon dioxide ($CO_2$) produced from buffering and fermentation. Diets contained ryegrass and 0, 15, 35 or 50% DM of fodder beet root (0FB, 15FB, 35FB, 50FB). Significance diet interactions are denoted by * $P$ <0.05, ** $P$ <0.01, *** $P$ <0.001.

contribution to exogenous protein supply is still needed, as an inverse relationship exists between microbial biomass production and the gas produced from the fermentation of carbohydrates (Blümmel et al., 1997; Cone and van Gelder, 1999; Guyader et al., 2016).

The decline of branched-chain VFA in response to the increased FB supply reflects the lower supply of protein and amino acids in FB compared with pasture. While FB reduced the N available, the proportion in substrate still exceeded the 25 mg N/g substrate required for microbial activity (Mould et al., 2005). Branched-chain fatty acids, including; iso-butyrate and iso-valerate, are produced from microbial fermentation of valine and leucine residues, respectively (El-Shazly, 1952; Allison, 1978).

There is a positive correlation between the dietary supply of protein and subsequent branched-chain VFA concentration of ruminal fluid (Annison, 1954). The observed decline of branched-chain VFA implies the reduced dietary supply of valine and leucine amino acids. However, further work is required to define the effect of FB on microbial biomass, community profiles and exogenous protein synthesis *in vivo*.

## 6.6 Conclusion

Analysis of incubation fluid suggests the percentage of FB in a perennial ryegrass-based diet increased the rate of fermentation and the formation of butyrate while reducing the concentrations of branched-chain fatty acids. Fodder beet was also found to increase propionate concentrations which reduced $CH_4$ fractions in gas. While the low N content of FB was probably not limiting for microbial metabolism, further examination of the inhibitory effect of microbial protein synthesis on enteric gas production from fodder beet is required *in vivo*. The results suggest that supplementation of a perennial ryegrass-based diet consisting of greater than 35% FB root increases the risk of ruminal acidosis *in vivo* due to the faster rate of fermentation than perennial ryegrass herbage. The volume of $CH_4$ and $CO_2$ per g DM of the substrate (from fermentation and buffering) increased relative to the proportion of FB due to the greater OM content compared with herbage. Therefore, supplementing herbage with FB root may not reduce enteric $CH_4$ emissions when FB allocation is < 35% of DMI, although further investigation *in vivo* is required to confirm this finding.

# Chapter 7

# Modelling feeding strategies to improve milk production, rumen function and discomfort of the early lactation dairy cow supplemented with fodder beet.

*Published in the Journal of Agricultural Science (July 2020)*

## 7.1 Abstract

New Zealand dairy producers have recently adopted fodder beet (FB) to supplement the early-lactation herbage supply, despite the limited definition of feeding and grazing management practices which may prevent acute and sub-acute ruminal acidosis (SARA). This modelling study aimed to characterise changes of rumen pH, milk production and total discomfort from FB and define practical feeding strategies of a mixed herbage and FB diet. The deterministic, dynamic, and mechanistic model, MINDY, was used to compare a factorial arrangement of FB allowance, herbage allowance (HA), and allocation time. The FB allocations were 0, 2, 4 or 7 kg DM/cow per day (0, 2, 4 and 7FB, respectively) and HA were 18, 24 or 48 kg DM/cow per day above ground. All combinations were offered either in the morning or afternoon or split across two equal meals. Milk production from 2FB diets was similar to control but declined 4, and 16% when FB increased to 4 and 7 kg DM, respectively. MINDY predicted that 7FB would result in SARA and that rumen conditions were sub-optimal even at moderate FB allocations (pH < 5.6 for 160 and 90 min/d, 7 and 4FB respectively). Pareto Front analysis identified the best compromise between high milk production and low total discomfort was achieved by splitting the 2FB diet into two equal meals fed each day with 48 kg DM of herbage. However, due to low milk response and high risk of acidosis, we conclude that FB is a poor supplement for lactating dairy cows.

## 7.2 Introduction

In the last five years, fodder beet (*Beta vulgaris L.*; FB) has been widely adopted as a winter forage crop or as a supplement during early- and late-lactation to ryegrass-based (*Lolium perenne*) pastoral dairy systems of New Zealand. While the use of FB has declined slightly within the last two years, the popularity of FB has previously been driven by the high yield

potential > 20 t DM/ha (Chakwizira et al., 2013; Chakwizira et al., 2016), high crop utilisation > 90% (Saldias and Gibbs, 2016), and the versatility to graze FB in autumn and winter or to harvest bulb and supplement herbage in spring. Fodder beet is rich in water-soluble carbohydrate (WSC) which accounts for over 60% of the biomass (Clark et al., 1987) and contains minimal proportions of fibre (< 20% DM) and crude protein (CP: < 10%) (Dalley et al., 2017). The low CP and high WSC content of FB bulb may reduce N excretion by diluting intake of high CP herbage (Dalley et al., 2019), or by synchronising the supply of WSC to soluble protein. Nutrient synchrony may improve microbial utilisation of soluble protein and reduce the excretion of urea in urine, which contributes to eutrophication and N leaching (Hall and Huntington, 2008; Cameron et al., 2013). However, the large fraction of sugar and low fractions of fibre also present a risk for sub-acute (SARA) and acute ruminal acidosis (Owens et al., 1998). Acidosis occurs when volatile fatty acids (VFA) rapidly accumulate in the rumen, causing pH to decline and limiting microbial degradation of cellulose and fibre (Khafipour et al., 2009). Subacute ruminal acidosis is characterised by daily episodes of low pH and reduced buffering capacity (Owens et al., 1998), but is self-corrected. Declining pH causes the proliferation of microbes that produce lactic acid, which is 10-fold more acidic than VFA (Owens et al., 1998). Increased lactic acid causes a downward spiral of rumen pH leading to acute and often systemic acidosis that the animal cannot self-correct.

While acute presentation represents an immediate loss of capital to the dairy business, monetary losses from SARA are not obvious but maybe equally financially harmful due to the limited diagnostic ability and reported wide-spread prevalence across the herd (Plaizier et al., 2008). Cows that suffer from SARA experience welfare challenges such as reduced intake, malaise and translocation of endotoxin present in the cell wall of gram-negative bacteria, which can cause liver abscesses, systemic inflammation and laminitis (Nagaraja et al., 1978; Nocek, 1997; Gozho et al., 2005; Zebeli and Metzler-Zebeli, 2012b). Low ruminal pH may also limit the lifetime productivity of the cow due to residual effects to the rumen epithelium (keratinisation) that reduce the absorption of VFA from the rumen and further increase susceptibility to acidosis (Kleen et al., 2003). Mean ruminal pH has been identified as a poor technique for defining SARA. While the duration of pH below a threshold of 5.8 (Zebeli et al., 2008) or 5.6 (Gozho et al., 2005), better describe the tangible effects to microbial activity, there is no singular agreed-upon threshold across the literature due to variation of response

among individual animals. Consequently, alternative indicators of animal welfare, other than episode duration of low pH, are needed.

One option to assess welfare is to quantify the 'discomfort' of livestock. Minimal total discomfort is the additive integration of internal (due to changes of internal state and supply nutrients) and external signals (changes of the environment) by the central nervous system (Forbes, 1996; Forbes and Provenza, 2000). The connection of taste and visceral afferents in the solitary nucleus with the limbic system in the cerebral cortex, allows ruminants to adjust feeding behaviour to suit momentary nutrient demands (Provenza, 1995). For example, ruminants may choose to select nutritious foods (positive reinforcement) and avoid toxins (negative reinforcement) based on additive post-ingestive feedbacks (Forbes, 1996; Provenza, 1996). Furthermore, foods such as FB, which are toxic, deficient in nutrients or rich in readily digestible nutrients, are likely to cause stronger aversions than feeds such as pasture (Forbes, 2007a). While SARA will cause increased discomfort and aversions to FB, feeding management involving a combination of timing, frequency and DM allocation, of both herbage and FB may help to alleviate discomfort and improve animal welfare.

The effect of FB on ruminal pH has been reported for beef cattle. Feeding FB *ad libitum* to beef steers in metabolism crates did not affect mean pH compared with animals fed a traditional ryegrass herbage diet (Prendergast and Gibbs, 2015). However, Waghorn et al. (2018) reported 5 out of 8 non-lactating dairy cows, developed acute acidosis, when similar FB allocations were fed. Despite the increased use of FB as an early lactation supplement to utilise residual winter forage, research of the effects on early lactation rumen function and fermentation is scarce. Waghorn et al. (2019) reported acidosis in two out of four late lactation dairy cows fed a diet containing 60% FB and industry recommendations for lactating cows are < 40% dietary inclusion. However, the rising plane of nutrition experienced early in lactation is a key driver of acidosis, due to the greater nutrient demand that is experienced postpartum and the risk of SARA may be greater than during late-lactation (Penner et al., 2007). Definition of the amount and frequency of FB and herbage fed during early lactation, and the potential impact to animal health and production is required.

The primary objective of this modelling study was to investigate how DM allocation of FB in early lactation affected ruminal pH and total discomfort when used to supplement the spring herbage supply. The secondary objective was to explore whether a feeding strategy, as

a factorial arrangement of time of day, amount and frequency of herbage and FB allocation, could improve total discomfort and milk production of early lactation dairy cows supplemented with FB.

## 7.3 Materials and methods

### 7.3.1 Model description

MINDY is a deterministic, mechanistic and dynamic model of a grazing ruminant that simulates diurnal metabolism patterns by assessing animal internal state and external motivations to feed. The model consists of seven component models which include: (1) the dairy cow digestion and metabolism model of (Baldwin, 1995) which was modified by Gregorini et al. (2013) and models of (2) diurnal grazing patterns and feed motivation, (3) sward structure and herbage chemical composition, (4) grazing behaviour, (5) dietary preference and selection (Gregorini et al., 2015), (6) grazing bioenergetics oral processing and digesta outflow (Gregorini et al., 2018b), and (7) a model of diurnal urination and drinking patterns (Gregorini et al., 2018a). Equations, coding, model validation, and sensitivity analysis of MINDY have been previously reported (Gregorini et al., 2013; Gregorini et al., 2015; Gregorini et al., 2018b; Gregorini et al., 2018a)

### 7.3.2 Simulation design

We simulated 90 dietary treatments using a factorial arrangement of FB allowance (FBA), herbage allowance (HA), and allocation time. The allocations of FB bulb were 0, 2, 4 and 7 kg DM/cow per day (0FB, 2FB, 4FB and 7FB respectively) and herbage allocations were 18 (75$m^2$/cow), 28 (115 $m^2$/cow) and 48 kg DM/cow (200 $m^2$/cow) per day above ground (18HA, 28HA and 48HA, respectively). Based on the expected post-grazing residual of 1550 kg DM/ha, MINDY was allocated 10.5 (18HA), 16.1 (28HA) and 28 kg DM/day (48 HA) of available herbage mass per day. In the simulations, herbage was offered either in the morning (AM) or afternoon (PM) or split across two equal meals following morning and afternoon milking (AM+PM). Fodder beet was also fed following morning (AMS), afternoon (PMS) or morning and afternoon milking (A+PS). MINDY was initialised as a four-year-old Frisian X Jersey dairy cow between 30-50 days of lactation with an initial liveweight of 533 kg. Milking was scheduled for 0600 and 1600 h each day. MINDY was set to record all outputs every 15 min over 20 days, although data obtained from the initial ten days were removed to ensure model stabilisation.

The nutritional composition of herbage was based on a typical rotationally grazed spring perennial ryegrass dominant sward, grazed at an extended tiller height of 30 cm (2900 kg DM/ha). The chemical composition of pasture and FB are shown in Table 7.1. Fodder beet consumption simulated a harvested bulb (defoliated during harvesting), which was offered on a feed pad following milking. The time spent on the fed pad was simulated to increase with the amount of FB allocated (15, 60 and 120 min/day, 2, 4 and 7FB respectively) and which enabled MINDY to finish the FB meal. The time spent on the feed pad increased with FBA because of the declining attraction to supplement that reflects the specific satiety parameter described by Gregorini et al. (2015). Briefly, attraction to a specific feed increases with time passed since the last meal and declines as the feed is eaten.

### 7.3.3 Outputs and analysis

Outputs requested of the model were: dry matter intake (DMI) of herbage and FB (kg DM/cow/day), milk production and composition (kg and percentage of fat and protein) and nitrogen (N) concentration of faeces and urine. Diurnal variations of rumen fermentation products (ammonia, pH, acetate, butyrate, propionate, lactate and total volatile fatty acid concentrations; VFA), rumen DM pool, neutral detergent fibre (NDF), acid detergent fibre (ADF), ruminal passage of organic matter (OM), and total discomfort, were also requested. Total discomfort is a parameter derived from the minimum total discomfort model of Forbes (2007b) and integrated into MINDY (Gregorini et al., 2015).

The first step involves calculating momentary optimal feed (MOF) based on optimal feed and MINDY's momentary internal state. Where momentary refers to any particular point in time and optimum is a set of defined conditions. According to MINDY's current internal state, the MOF is then determined by adjusting each macronutrient up or down from the optimum value. For every percentage point deviation from the standard levels, e.g. from the defined ruminal pH of 6.2, an increase or decrease percentage of particular macronutrient is applied by a linear factor. Linear factors of ruminal pH, ammonia concentrations, NDF, ME, and hunger, are calibrated so that the balance of one driver at a time restores or brings MINDY close to the 'standard levels'. Thus, a meal of MOF will restore MINDY's comfort. The model is then asked to calculate Total Discomfort from a particular feed against the MOF and is a unitless value. Therefore, the greater the Total Discomfort, the lower the attraction to a particular feed.

$$Total\ Discomfort = \sqrt{\sum_{j=1}^{i} wj \left[\left(\frac{oj - cj}{oj}\right)\right]^2} \qquad (6.1)$$

Briefly, *w*, *c* and *o* represent the weighting of the current and momentary optimal supply of nutrient *j* of the set of *i* nutrients. We assumed that MINDY follows a set of rules and makes the 'correct' decision based on her internal state, which has been reported previously (Gregorini et al., 2015).

Differences of model outputs do not vary within each simulation; thus, statistical analysis is not possible. Therefore, diet and time effects are discussed in terms of absolute differences and not statistical significance. Outputs were averaged across days and between each step of data collection. The step size of ruminal pH and rumen fermentation end-products were collected every 15 min, and data points were also averaged across the day. Milk production, DMI, nitrogen excretion were also requested each day. Data were averaged across the medium HA allocation fed either AM or PM but not AM+PM, to determine the effect of FBA on ruminal pH and total DMI. Diets were screened using a multi-objective optimisation technique called the Pareto front analysis, a computer-based decision support system that identifies scenarios that are the 'best' trade-off in outcomes (Matthews et al., 2002). Diets which maximised milk production and reduced total discomfort were defined as optimal solutions. Optimal solutions are known as the Pareto front which will be herein referred to as the Pareto frontier and was conducted using the function *psel* in the R (R Core Team, 2018, v. 3.4.4) package *rPref (http://cran.r-project.org/web/packages/rPref/index.html).*

Table 7.1. Chemical composition of herbage and Fodder beet (FB) bulb.

| Variables | Herbage | FB bulb |
|---|---|---|
| Pre-grazing Herbage mass (kg DM/ha) | 2904 | - |
| DM[1] % | 19.0 | 16.3 |
| CP[2] % | 22.4 | 7.00 |
| Lipid % | 4.13 | 5.16 |
| Starch % | 5.25 | 1.03 |
| WSC[3] % | 17.6 | 72.0 |
| NDF[4] % | 37.8 | 10.7 |
| ADF[5] % | 24.8 | 5.70 |
| Lignin % | 4.81 | 2.00 |
| Ash % | 9.03 | 4.32 |
| ME[6] (MJ/kg DM) | 10.5 | 11.9 |
| Soluble CP[7] % | 56.0 | 0.20 |
| RUP[8] % | 25.0 | 0.40 |
| NPN[9] % | 1.90 | 0.00 |

[1] Dry matter, [2] crude protein, [3] water-soluble carbohydrate, [4] neutral detergent fibre, [5] acid detergent fibre, [6] metabolisable energy [7] % of CP that is soluble, [8] % of CP that is rumen undegradable protein, [9] % of CP that is non-protein nitrogen.

## 7.4 Results

### 7.4.1 Intake and milk production

Across all herbage allocations, FB represented 0, 13.0, 26.6, and 57.1% of predicted daily DMI (0, 2, 4, and 7FB, respectively). Herbage intake declined in response to FBA by 2.3% (2FB), 30% (4FB), and 79% (7FB), compared with FB0. Compared with 0FB, 2FB increased DMI by 8.3%, 4FB maintained DMI, but 7FB reduced DMI by 28%. Feeding MINDY fodder beet A+PS increased DMI by 17.2% in 4FB and 31.4% in 7FB, compared with once-daily feeding. Within the 2FB diet, PM allocation of herbage increased DMI between 1-2% across all HA, and DMI responded similarly to PM allocation of both FB and herbage, although differences are not substantial (Figure 7.1). Compared with 18HA, MINDY predicted DMI of the 0FB treatment would increase 18% when allocated 28HA and 33% when allocated 48HA (13.1, 15.5 and 19.5 kg DM/d, 18HA, 28HA and 48HA respectively). Within the 0FB diet, PM allocation of herbage did not affect DMI when compared with AM allocation although, AM+PM grazing reduced DMI (15.3, 15.1 and 14.1 kg DM/cow/d, respectively). Predicted DMI was greatest (19.7 kg DM/cow) when 4FB was fed A+PS with 48HA allocated AM or PM and lowest (9.4 kg DM/cow) when 7FB as fed AMS with 18HA allocated AM+PM (Figure 7.1). Substitution rate declined

when FB was fed A+PS compared with AMS or PMS feeding (Figure. 6.2). Across all FB supplementation levels, AM+PM grazing reduced total DMI compared with AM or PM grazing (Figure 6.1).

Model predictions of milk production reflected DMI which compared with 18HA increased 7.1 (28HA) and 15.8% (48HA) (24.0, 26.3 and 22.4, L/cow/day, 18, 28 and 48HA, respectively) although, the effect of HA on milk solids yield was less pronounced (Figure 7.1). When the time of FBA allocation is ignored, MINDY predicted a curvilinear response to FBA, as milk yield increased 2.5 % with 2FB and declined 8.2 and 19.8% with 4FB and 7FB, respectively, compared with 0FB (Figure 7.1). However, milk production increased when FB was fed A+PS compared with AMS or PMS feeding (1.6 %, 12.2% and 13.4%, 2FB, 4FB and 7FB, respectively), while AMS and PMS feeding produced similar quantities of milk (Figure 7.1). The lowest milk yield 18.2 L/cow occurred when 7FB was fed AM with 18HA, allocated AM+PM (Figure 7.2). While greatest milk yield 26.6 L/cow was achieved by feeding 2FB AM and allocating 48HA either AM or PM. Furthermore, feeding FB in the afternoon rather than the morning improved milk yield by 0.5 kg/d, irrespective of FBA. The predicted milk response to FB (kg milk/kg DM of FB) increased between 2FB and 4FB but declined at greater FBA. The milk response to FB increased when herbage and FB were fed in the afternoon, and when HA was restricted (Figure 7.2). The percentage of milk solids decreased with increased FBA (10, 10, 9, and 8 %, 0, 2, 4 and 7FB, respectively), reflecting reduced proportions of both protein and fat. Total yields of milk fat and protein declined with greater DM allocation of FB (Table 7.2). Herbage allowance did not affect milk solids yield, other than a slight increase in milk fat (Table 7.2).

### 7.4.2 Ruminal pH

The predicted daily mean of ruminal pH was not affected by FBA. However, differences were pronounced when the diurnal variation of pH was considered. The nadir pH across all HA declined with increased FB inclusion (5.83, 5.68, 5.40, and 5.34 pH, 0, 2, 4, 7FB, respectively), while zenith pH increased slightly with FBA (6.27, 6.25, 6.31 and 6.37 pH, 0, 2, 4, 7FB, respectively). Model predictions suggest that 0FB and 2FB diets would maintain ruminal pH above 5.6 (Table 7.3). Moderate and large allocation of FB increased the duration of pH < 5.6 (~90 and 160 min/day, 4FB and 7FB respectively), which was corrected by increasing the frequency that FB was fed (Figure 7.3) as rumen pH > 5.6 was maintained when 4FB was fed A+PS. Twice-daily feeding of FB also reduced the time that pH was < 5.6 in 7FB diets by ~ 17

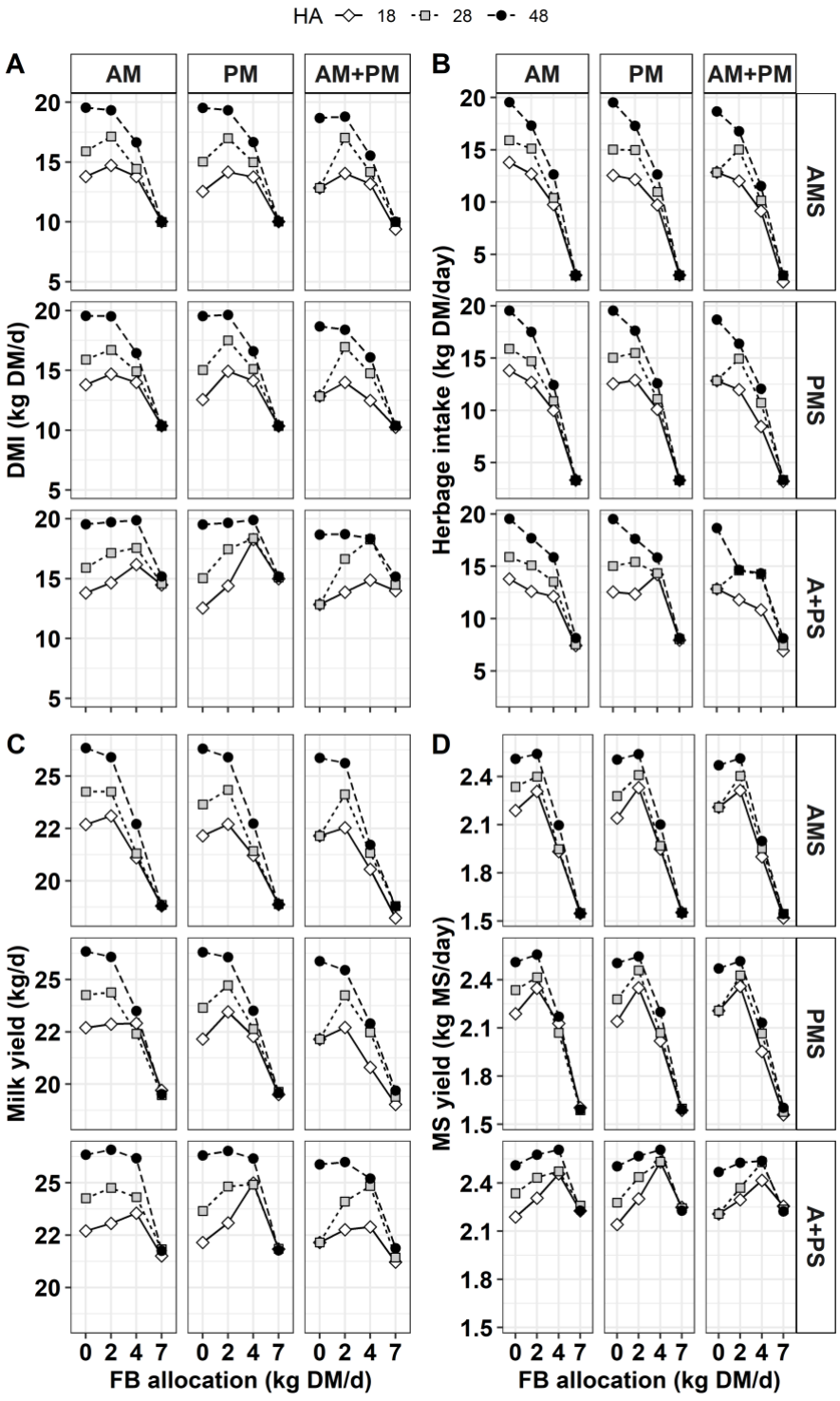


Figure 7.1. (A) Daily intake of dry matter (DMI) and (B) herbage (kg DM/cow), (C) daily milk yield ( kg/cow) and (D) milk solids (fat + protein) yield (MS: kg/cow) in response to allocation of fodder beet (FB) fed either in the morning (AMS), afternoon (PMS) or morning and afternoon (A+PS), and herbage allocation (HA: 18, 28, 48 kg DM/cow/day) fed in the morning (AM), afternoon (PM) or morning and afternoon (AM+PM).

Table 7.2. Predicted milk fat and protein (kg/cow/day) of cows fed fodder beet (FB) fed either in the morning (AMS), afternoon (PMS), or morning and afternoon (A+PS) and varying herbage allocations fed morning (AM), afternoon (PM) or morning and afternoon (AM+PM).

| | | | **0FB[1]** | **2FB** | | | **4FB** | | | **7FB** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **HA[2]** | Time | None | AMS | PMS | A+PS | AMS | PMS | A+PS | AMS | PMS | A+PS |
| **Milk fat (kg/day)** | **18HA** | AM | 1.41 | 1.51 | 1.56 | 1.51 | 1.21 | 1.34 | 1.65 | 0.91 | 0.94 | 1.49 |
| | | PM | 1.38 | 1.55 | 1.54 | 1.51 | 1.22 | 1.25 | 1.67 | 0.91 | 0.93 | 1.50 |
| | | AM+PM | 1.44 | 1.54 | 1.58 | 1.51 | 1.19 | 1.24 | 1.63 | 0.90 | 0.92 | 1.53 |
| | **28HA** | AM | 1.50 | 1.56 | 1.57 | 1.58 | 1.22 | 1.30 | 1.63 | 0.91 | 0.93 | 1.51 |
| | | PM | 1.46 | 1.57 | 1.61 | 1.58 | 1.23 | 1.29 | 1.67 | 0.91 | 0.93 | 1.49 |
| | | AM+PM | 1.44 | 1.57 | 1.59 | 1.54 | 1.22 | 1.29 | 1.67 | 0.91 | 0.92 | 1.50 |
| | **48HA** | AM | 1.60 | 1.65 | 1.66 | 1.66 | 1.32 | 1.36 | 1.71 | 0.91 | 0.93 | 1.48 |
| | | PM | 1.60 | 1.65 | 1.65 | 1.65 | 1.32 | 1.39 | 1.70 | 0.91 | 0.93 | 1.48 |
| | | AM+PM | 1.58 | 1.63 | 1.64 | 1.63 | 1.25 | 1.34 | 1.67 | 0.91 | 0.94 | 1.47 |
| **Milk protein (kg/day)** | **18HA** | AM | 0.78 | 0.80 | 0.79 | 0.79 | 0.72 | 0.79 | 0.81 | 0.64 | 0.67 | 0.74 |
| | | PM | 0.76 | 0.78 | 0.81 | 0.80 | 0.73 | 0.77 | 0.86 | 0.64 | 0.66 | 0.75 |
| | | AM+PM | 0.76 | 0.78 | 0.78 | 0.78 | 0.71 | 0.71 | 0.79 | 0.62 | 0.64 | 0.73 |
| | **28HA** | AM | 0.84 | 0.84 | 0.84 | 0.85 | 0.73 | 0.77 | 0.84 | 0.64 | 0.66 | 0.75 |
| | | PM | 0.82 | 0.84 | 0.85 | 0.86 | 0.74 | 0.78 | 0.86 | 0.64 | 0.66 | 0.75 |
| | | AM+PM | 0.76 | 0.83 | 0.84 | 0.83 | 0.73 | 0.77 | 0.86 | 0.64 | 0.66 | 0.74 |
| | **48HA** | AM | 0.91 | 0.89 | 0.90 | 0.92 | 0.78 | 0.81 | 0.90 | 0.64 | 0.66 | 0.75 |
| | | PM | 0.91 | 0.89 | 0.90 | 0.91 | 0.78 | 0.81 | 0.90 | 0.64 | 0.66 | 0.75 |
| | | AM+PM | 0.89 | 0.88 | 0.88 | 0.90 | 0.75 | 0.79 | 0.87 | 0.64 | 0.67 | 0.75 |
| **Milk solids (kg/day)** | **18HA** | AM | 2.19 | 2.31 | 2.35 | 2.31 | 1.93 | 2.13 | 2.46 | 1.55 | 1.60 | 2.23 |
| | | PM | 2.14 | 2.33 | 2.35 | 2.30 | 1.95 | 2.02 | 2.53 | 1.55 | 1.59 | 2.25 |
| | | AM+PM | 2.21 | 2.31 | 2.36 | 2.30 | 1.90 | 1.95 | 2.42 | 1.52 | 1.56 | 2.26 |
| | **28HA** | AM | 2.34 | 2.40 | 2.42 | 2.43 | 1.95 | 2.07 | 2.47 | 1.55 | 1.59 | 2.26 |
| | | PM | 2.28 | 2.41 | 2.46 | 2.44 | 1.97 | 2.07 | 2.53 | 1.55 | 1.60 | 2.24 |
| | | AM+PM | 2.21 | 2.40 | 2.43 | 2.37 | 1.95 | 2.07 | 2.53 | 1.55 | 1.58 | 2.24 |
| | **48HA** | AM | 2.51 | 2.54 | 2.56 | 2.58 | 2.10 | 2.17 | 2.61 | 1.55 | 1.59 | 2.23 |
| | | PM | 2.50 | 2.54 | 2.54 | 2.57 | 2.10 | 2.20 | 2.61 | 1.55 | 1.59 | 2.23 |
| | | AM+PM | 2.47 | 2.51 | 2.52 | 2.53 | 2.00 | 2.13 | 2.54 | 1.54 | 1.60 | 2.22 |

[1]0FB: control no FB diet, 2FB: 2 kg DM FB, 4FB: 4 kg DM FB, 7FB: 7 kg DM FB/cow. [2]daily herbage allocation (HA) 18, 28, 48 kg DM/cow above ground, 18, 28 and 48HA, respectively

Table 7.3. Daily duration of pH <5.8 or 5.6 in response to varying allocations of herbage (HA) fed in the morning (AM), afternoon (PM) or morning and afternoon (AM+PM), and fodder beet (FB) fed morning (AMS), afternoon (PMS) or morning and afternoon (A+PS).

| | | | **0FB[1]** | **2FB** | | | **4FB** | | | **7FB** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **HA[2]** | **HA Time** | None | AMS | PMS | A+PS | AMS | PMS | A+PS | AMS | PMS | A+PS |
| | | AM | 0 | 135 | 45 | 0 | 150 | 150 | 210 | 255 | 255 | 255 |
| | **18HA** | PM | 0 | 60 | 150 | 30 | 150 | 165 | 210 | 255 | 255 | 255 |
| | | AM+PM | 0 | 105 | 120 | 0 | 150 | 180 | 225 | 255 | 270 | 285 |
| | | AM | 0 | 120 | 60 | 0 | 150 | 150 | 225 | 255 | 255 | 270 |
| pH <5.8 (min/d)[3] | **28HA** | PM | 0 | 60 | 150 | 0 | 150 | 150 | 195 | 255 | 255 | 270 |
| | | AM+PM | 0 | 120 | 150 | 0 | 150 | 150 | 240 | 255 | 255 | 270 |
| | | AM | 0 | 30 | 60 | 0 | 150 | 150 | 195 | 255 | 255 | 255 |
| | **48HA** | PM | 0 | 30 | 60 | 0 | 150 | 150 | 195 | 255 | 255 | 255 |
| | | AM+PM | 0 | 60 | 60 | 0 | 150 | 150 | 255 | 255 | 255 | 255 |
| | | AM | 0 | 0 | 0 | 0 | 90 | 75 | 0 | 165 | 165 | 150 |
| | **18HA** | PM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 180 | 150 |
| | | AM+PM | 0 | 0 | 0 | 0 | 90 | 105 | 0 | 180 | 180 | 150 |
| | | AM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 180 | 135 |
| pH <5.6 (min/d)[4] | **28HA** | PM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 180 | 135 |
| | | AM+PM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 180 | 135 |
| | | AM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 180 | 135 |
| | **48HA** | PM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 180 | 135 |
| | | AM+PM | 0 | 0 | 0 | 0 | 90 | 90 | 0 | 165 | 165 | 135 |

[1]0FB: control no FB diet, 2FB: 2 kg DM FB, 4FB: 4 kg DM FB, 7FB: 7 kg DM FB/cow.

[2]daily herbage allocation (HA) 18, 28, 48 kg DM/cow above ground, 18, 28 and 48HA, respectively. [3]Daily duration of rumen pH <5.8, [4] Daily duration

min/day. Consideration of time at which pH < 5.8 provides further definition of the effect of FBA on diurnal patterns of rumen pH. MINDY predicted that time pH < 5.8 would also increase relative to FBA (pH <5.8: 0, 59.4, 173.9, 258.3 min/d). However, A+PS feeding increased the time pH was <5.8 compared with once-daily feeding of 4 and 7FB (Table 7.3)

### 7.4.3 Rumen fermentation and outflows of digesta

MINDY predicted that rumen concentrations of VFA would increase with HA (0.10, 0.11 and 0.12 mol/L, 18, 28 and 48HA, respectively), and model predictions were minimally affected by the time of HA (< 1.25 %). Mean VFA concentration declined when 7 FB was fed, compared with smaller allocations of FB (0.107, 0.121, 0.118 and 0.087 mol/L, 0, 2, 4 and 7FB, respectively). Morning and evening feeding of 4FB and 7FB caused VFA concentrations to increase (2-3-fold), 2-3 h following FB consumption (Figure 7.3). Minor increases of propionate were also detected in response to FBA, but propionate concentrations declined at the greatest FBA (0.023, 0.025, 0.026 and 0.019 mol/L, 0, 2, 4, 7FB, respectively). Model predictions indicate greater concentrations of acetate with 2FB but acetate concentrations declined as FBA increased (0.071, 0.81, 0.78 and 0.57 mol/L, 0, 2, 4 and 7FB, respectively). At moderate (4FB) and high allocations (7FB) of FB, the rumen concentration of lactic acid increased substantially (0.01 and 0.08 mol/L, 4FB and 7FB, respectively). However, lactic acid concentrations declined when FB was fed A+PS compared with either AMS or PMS feeding. There was a small positive interaction between FBA and the amount of herbage offered. However, lactic acid concentrations were negligible when 2FB or 4FB were fed A+PS.

Model predictions suggest the daily mean concentration of rumen ammonia increased in response to HA (0.016, 0.019 and 0.023 mol/L 18, 28 and 48HA, respectively) and declined when herbage was fed AM+PM. Minor differences of rumen ammonia were observed between AM or PM herbage allocation but declined with increasing FBA (Figure 7.3). Consumption of FB reduced ruminal ammonia concentrations although, only small differences were apparent between 2 and 4FB, while 7FB caused ammonia to decline (0.023, 0.022, 0.020 and 0.012 mol/L, 0, 2, 4, and 7FB respectively), which also reflect the reduced intake of herbage. The difference in rumen ammonia between AMS and PMS feeding of FB increased with FBA and ranged from 0.4-16.3%, reflecting herbage intake.

Predicted ruminal pools of NDF and ADF declined in response to FB intake (5.5, 5.4, 5.1 and 3.8 kg/day, 0, 2, 4 and 7FB) but increased in response to HA (4.5, 4.8 and 5.1 kg/day, 18,

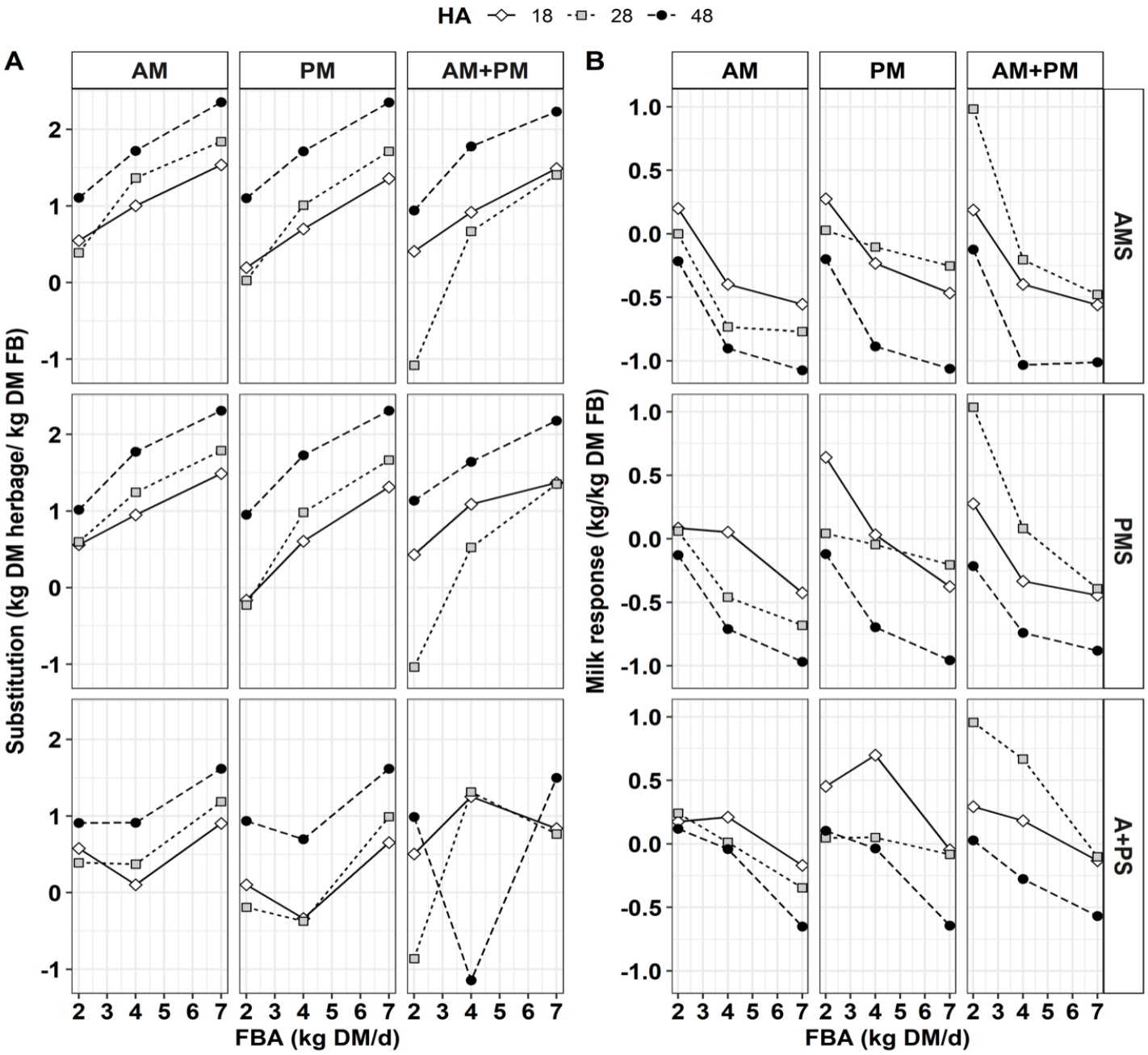


Figure 7.2. (A) Substitution rate (kg DM herbage/kg DM of FB) and (B) milk response (kg milk/kg DM FB) to increasing fodder beet allocation (FBA; 0, 2, 4, 7 kg DM/cow/day) fed morning (AMS), afternoon (PMS) or morning and afternoon (A+PS) and herbage allocation (HA: 18, 28, 48 kg DM/cow/day) fed in the morning (AM), afternoon (PM) or morning and afternoon (AM+PM).

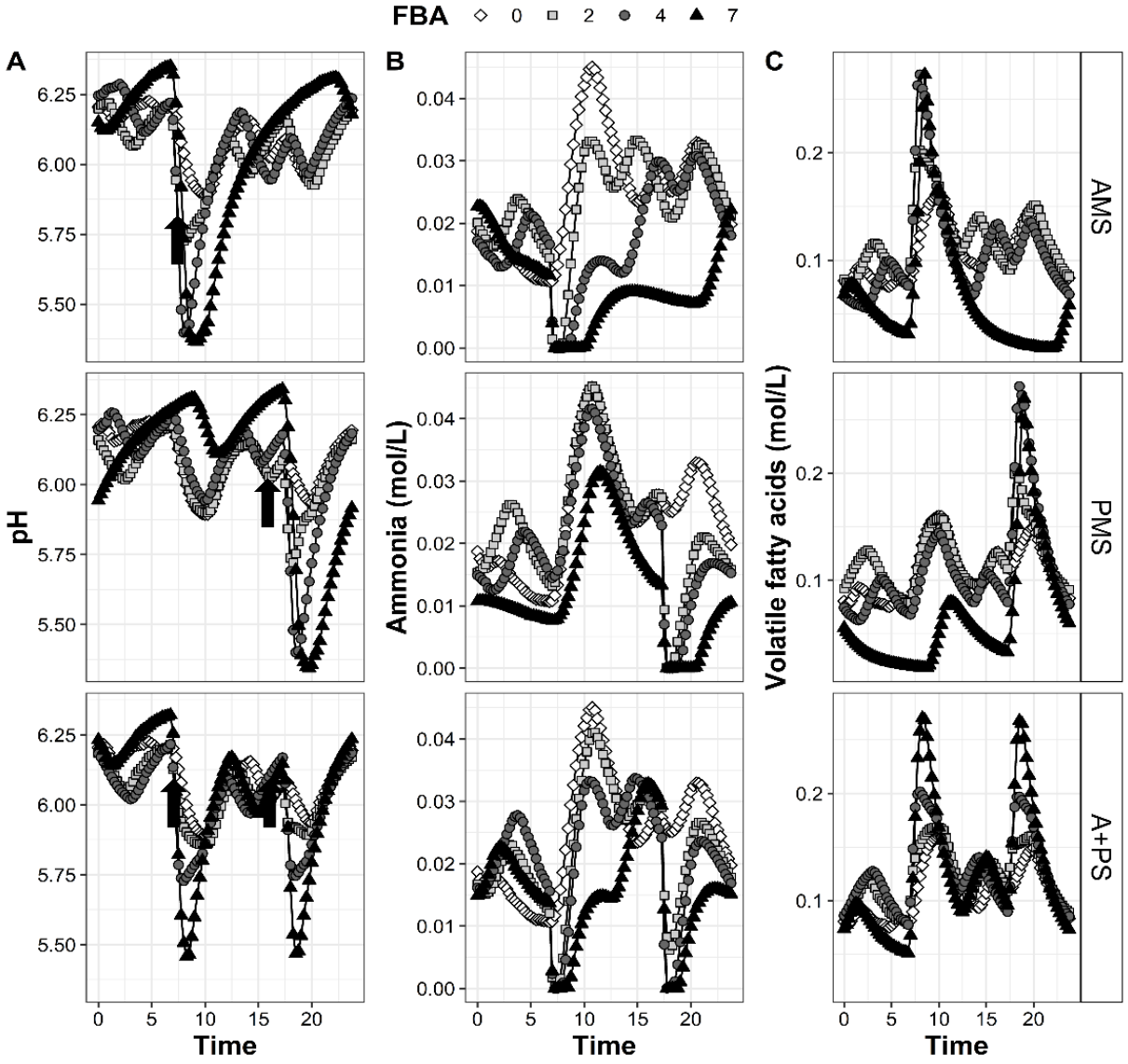


Figure 7.3. Diurnal variation of rumen pH (A), ammonia (B) and volatile fatty acid (C) concentrations (mol/L) when MINDY was fed 28 kg DM of pasture in the morning and different allocations of fodder beet (FBA) (0, 2, 4 or 7 kg DM of FB/cow/d) in the morning (AMS), afternoon (PMS) or evenly split over two meals morning and afternoon (A+PS). The arrow at the bottom of each section in the first column represent the time that FB was fed each day.

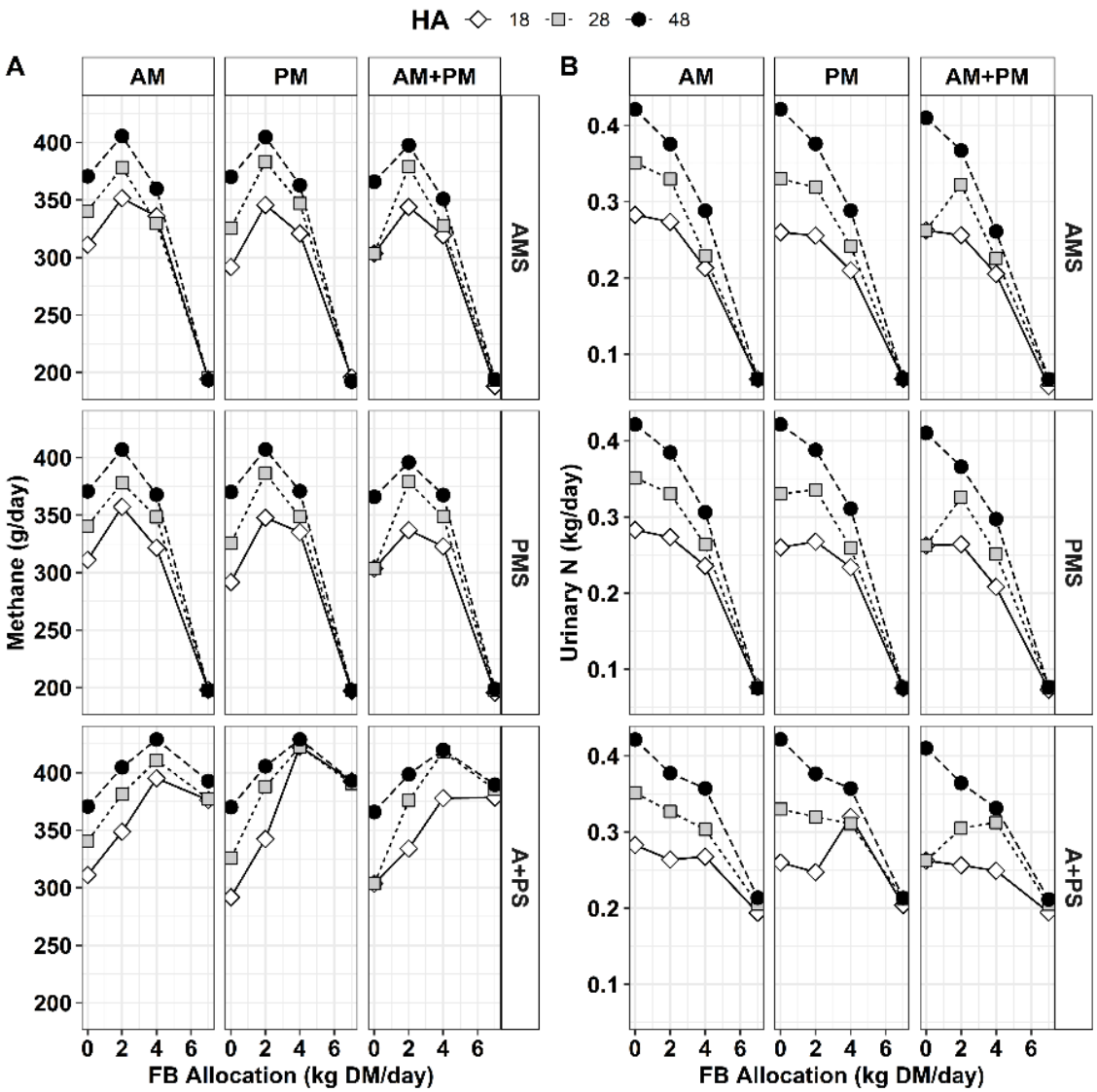


Figure 7.4. A: Methane emission (g /d) and B: urinary nitrogen excretion (kg/day) predicted from increasing fodder beet allocation (FBA; 0, 2, 4, 7 kg DM/cow/day) fed in the morning (AMS), afternoon (PMS) or morning and afternoon (A+PS) and herbage allocation (HA: 18, 28, 48 kg DM/cow/day) fed in the morning (AM), afternoon (PM) or morning and afternoon (AM+PM)

28, 48HA). Moderate and high allocations of FB reduced DM passage from the rumen below 0FB diets (10.9, 11.0, 10.0 and 7.1 kg/day, 0, 2, 4 and 7FB, respectively) and increased with the amount of herbage allocated (9.8, 10.6 and 12.3 kg/d, 18, 28 and 48HA kg/d, respectively). MINDY predicted the ruminal passage of DM would increase when FB was fed A+PS rather than AMS and PMS. When AMS and PMS rate of DM passage were averaged, twice-daily feeding of FB caused similar DM passage (-0.1 kg DM/d) at 2FB but increased DM passage by 1.3 kg/d at 4FB and 2.27 kg/d at 7FB.

Across the 28HA diets, the intake of N briefly increased and then declined, in response to greater intakes of FB (558, to 615, 476 and 238 g N per day, 0, 2, 4 and 7FB respectively). Morning and afternoon feeding of FB increased herbage intake and N compared with AMS or PMS feeding (548, 464 and 454 kg N/d, respectively); estimated N intake also increased with HA (484, 558 and 758 g N/day, 18, 28 and 48HA respectively). The low N content of FB bulb diminished urinary N excretion by 4 (2FB), 19 (4FB) and 65 % (7FB), compared with 0FB. Across all FB feeding rates, urinary N excretion increased when FB was fed A+PS compared with AMS or PMS feeding and increased with HA (Figure 7.4). While PM allocation of herbage slightly reduced the N content of urine (162, 139 and 83 g/d, 0, 2, 4 and 7FB, respectively), urinary N excretion increased with HA (125, 141 and 191 g N/d 18, 28 and 48HA, respectively). Faecal N content also declined with increased FB intake (141, 162, 139 and 83g/day; 0FB, 2FB, 4FB and 7FB, respectively) and increased with the amount of herbage allocated (125, 141 and 191g N/day; 18HA, 28HA and 48HA, respectively). Enteric methane emissions reflected DMI, increasing with HA and 2FB but decreasing with 4FB and 7FB diets. Feeding FB A+PS increased the $CH_4$ yield from 4FB and 7FB diets compared with AMS or PMS feeding (Figure 7.4). The Pareto frontier analysis, identified 7FB fed either AMS or PMS would provide the optimum solution for reducing pollutant swapping between $CH_4$ and urinary N/kg milk produced (Figure 7.5). Fodder beet allocation reduced urinary N intensity but increased the intensity of $CH_4$ emissions (Figure 7.5).

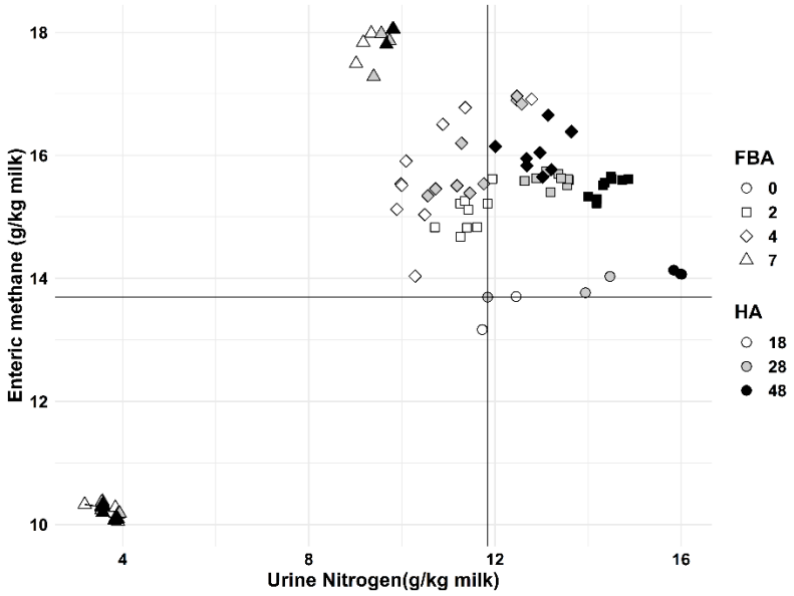


Figure 7.5. Pollution intensity of urinary nitrogen (g/kg milk/day) and enteric methane (g/kg milk/day) in response to fodder beet (FBA; 0, 2, 4 and 7 kg DM/cow/d) and herbage allocation (18, 28 and 48 kg DM/day). The 'frontier' (black line in the bottom left corner) represent diets which are the best compromise between reducing urinary nitrogen and enteric methane emissions. Diets along the frontier were all 7FB which caused sub-acute ruminal acidosis. The reference line represents a control 0FB diet with 28 kg DM/cow of herbage.

### 7.4.4 Total Discomfort

The combination of FB and herbage with time and frequency of allocation produced some feeding options, which increased milk yield and reduced animal discomfort. Total discomfort increased with FBA (17.1, 17.2, 17.3 and 17.6 units for 0, 2, 4 and 7FB respectively), but was not affected by the time or frequency of FBA or HA (Figure. 6.6). The Pareto front analysis identified seven diets which gave the optimal trade-off between milk production and discomfort (Figure 7.7). These diets consisted of four control (0FB) treatments of 28 and 48HA offered once daily, and one 48HA which was fed AM+PM. Of the seven diets, only two included FB, at 2FB fed twice daily with 48HA fed AM or PM.

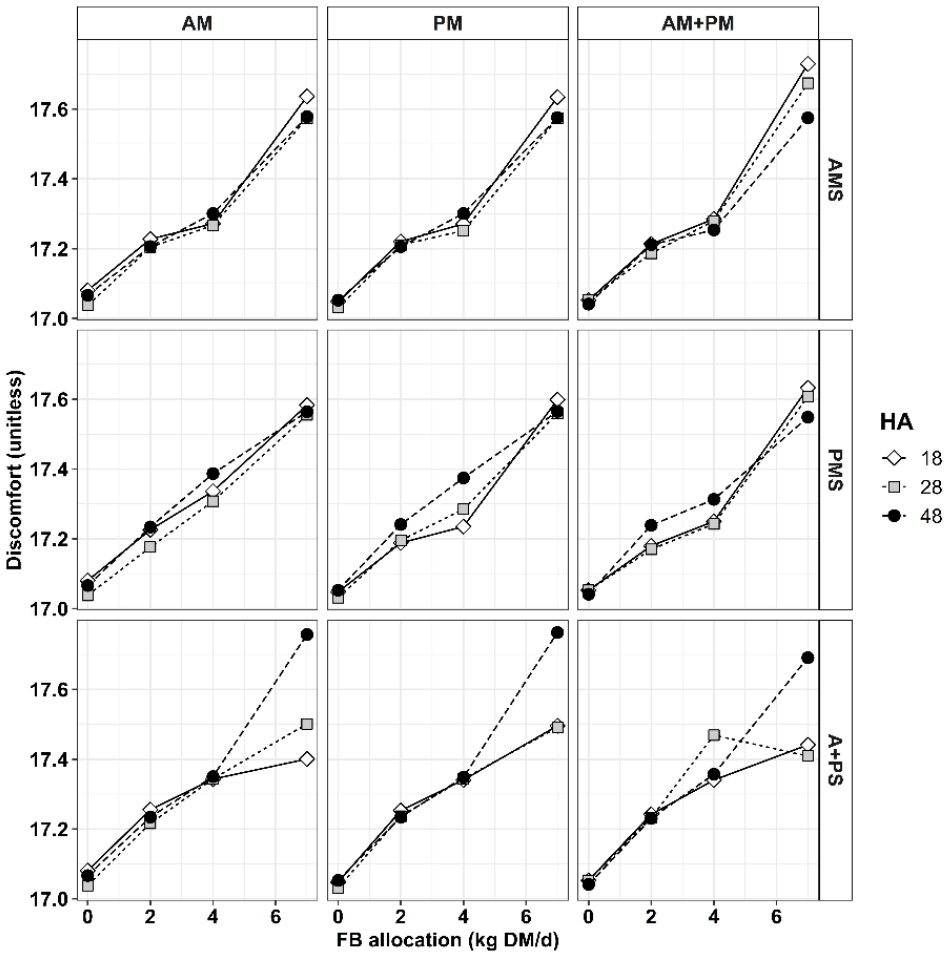


Figure 7.6. Total discomfort in response to increasing fodder beet allocation fed morning (AMS), afternoon (PMS) or morning and afternoon (A+PS) and herbage allocation (HA: 18, 28, 48 kg DM/cow/day) fed in the morning (AM), afternoon (PM) or morning and afternoon (AM+PM).

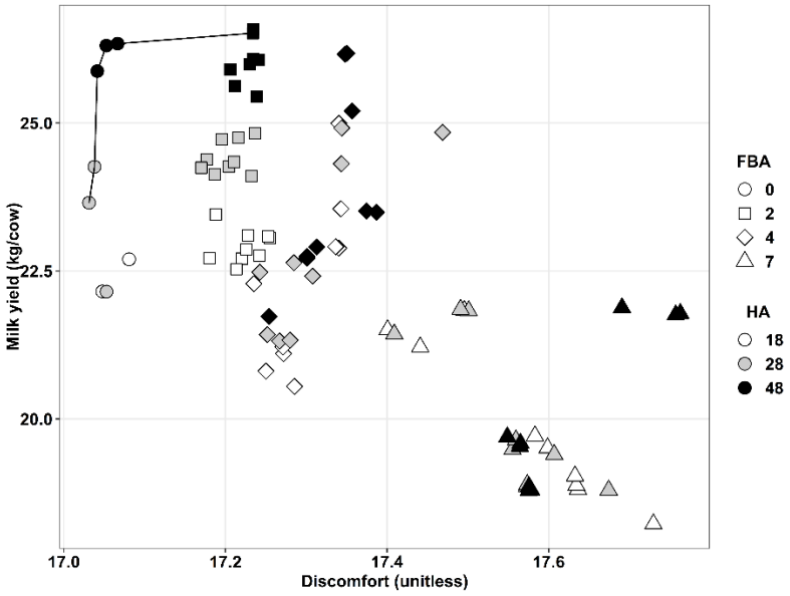


Figure 7.7. The relationship between fodder beet allocation (FBA; 0, 2, 4 and 7 kg DM/cow/d), milk yield (kg/cow/day) and discomfort. The 'frontier' (black line in the upper left corner) represent diets which are the best compromise between improving milk production and reducing animal discomfort.

## 7.5 Discussion

### 7.5.1 Ruminal pH and total discomfort

MINDY predicted a positive milk response to low FBA, but milk response and DMI declined at greater supplementation rates due to disruption of the rumen environment. MINDY predicted that the daily duration of pH < 5.6 would increase with FBA, causing moderate SARA from 4FB and 7FB diets. However, halving the FB meal reduced the duration of low rumen pH, compared with once-daily supplementation. These results agree with previous findings (Kaufmann, 1976; Cohen et al., 2006) and represent an increased distribution of soluble carbohydrate load in the rumen. While feeding FB A+PS increased the nadir pH, it was not completely able to correct the time that pH was < 5.6 (Gozho et al., 2005), which most likely reflects the increase of DM passage through the rumen (Waghorn et al., 2018). MINDY predicted that the 7FB treatment, which constituted 57% of total DMI, would result in a daily episodic decline of ruminal pH below 5.6 for 150 min/day. Predicted bouts of low pH reported here were longer in duration, and lower in value than those reported previously for beef steers fed *ad libitum* FB with 1 kg DM of lucerne silage (Prendergast and Gibbs, 2015). However, our values fit within the data obtained for lactating dairy cows consuming similar diets (Waghorn et al.,

2018). The time dependant threshold for SARA has been reported at pH < 5.6 for >180 min/day (Gozho et al., 2005), which was not met by either 7FB or 4FB diets in the modelling scenarios. However, the substantial increase of lactic acid concentration, reduced DMI and milk production indicate that MINDY experienced SARA when fed 7FB and that rumen conditions were sub-optimal when 4FB was fed.

Moderate allocations of FB (< 40% inclusion) have previously been reported as 'safe' for late-lactation dairy cows (Dalley et al., 2019; Waghorn et al., 2019). Discrepancies between late lactation studies and the current data set may be a consequence of cows being more susceptible to low pH during the postpartum transitioning period (Penner et al., 2007), but further *in vivo* studies are required to verify this finding. Diurnal variations of pH within the current data set appear to be accurate, as MINDY predicted pH would fall to the lowest values by around 3-4 hours after FB consumption, which agrees with experimental data (Waghorn et al., 2019). MINDY may have under-estimated the effect of FB on pH, as 60% dietary inclusion of FB caused clinical acidosis in late lactation (pH < 5.0) (Waghorn et al., 2019). Therefore, ruminal pH was expected to decline more when 7FB was fed, particularly under restricted grazing conditions. Model predictions strongly suggest that ruminal pH is below optimum when the meal size of FB exceeds 2 kg DM during early lactation, although twice daily feeding may help to stabilise the rumen environment by minimising the time that rumen pH is <5.6.

The total discomfort parameter of MINDY responded to a low ruminal pH and therefore, the amount of FB. The Pareto front analysis indicated a combination of low FBA fed twice daily, and high HA provided the best compromise between milk production and animal discomfort (Figure. 7.7). Supplementing a high HA with a small FB allocation is not a practical solution for NZ milk producers during a herbage deficit. Also, spring feeding of FB is a consequence of an excess of the winter crop which decomposes within three months once harvested. Therefore, feeding just 2 kg DM/d of FB may reduce the utilisation of supplement while, grazing high HA will reduce the nutritive composition of herbage and may require modified grazing management.

While MINDY predicted discomfort of the 'average' cow fed FB, consideration of social pressures affecting feeding motivation across the herd are also required. In the current scenario, MINDY represented a single individual, which was forced to complete her meal of FB by remaining on the feed pad until she had finished. There was a positive relationship

between time spent on the feed-pad and FBA, reflecting the increased discomfort experienced from FB as the meal progressed. Increased attraction to supplement preceding the meal and declining attraction as the meal progresses, is an aversive response to excessive supply of a nutrient (Forbes, 2007a) and reflects sensory inputs such as texture, odour, malaise or flavours (Provenza, 1995). However, in NZ pastoral systems, cows are commonly fed in herds rather than individually. Social hierarchies within the herd are known to restrict DMI of timid cows while enabling those who are more dominant to over-indulge (García et al., 2007). Timid cows are more susceptible to acidosis because they experience greater pressure by dominant animals to eat quickly and less frequently rather than eating based on signals of satiety or surfeit (eating to excess) (Owens et al., 1998). While MINDY does not predict the individual variation of eating behaviour and social hierarchies of the herd, further evaluation of this variation and its relationship with ruminal acidosis is needed.

To add further complexity, eating motivation and total discomfort changes considerably within individuals from day to day (Provenza, 1995; Gregorini et al., 2015). Overconsumption of FB will lead to an aversion due to the associated feeling of discomfort (Forbes and Provenza, 2000). An aversion to FB will increase the individual cow's risk of developing ruminal acidosis, as the variation of FB intake increases. Ruminants prefer to adjust their food choices to minimsze the feeling of discomfort (Provenza, 1995). However, choices are limited under pastoral grazing systems with harvested FB. Fodder beet is commonly fed-out on the paddock due to minimal use of infrastructure in NZ dairy systems. Spring feeding of FB in the paddock (before allocation of a new pasture break allocation) enables cows which experience discomfort from FB to choose between a supplement which increases total discomfort, grazing the residual pasture, or waiting until the new pasture break becomes available. Aversion of some individuals to FB will increase the risk of acidosis across the remaining herd, as the amount of FB allocated per cow increases. While it may be possible to identify and remove animals which are averse to eating FB, subtle variations of daily FB intake and SARA will not be visually detectable. Furthermore, as aversion is a negative reinforcement of post-ingestive feedback (Provenza, 1995), the animal's removal is not a preventative technique. Each animal should be provided with sufficient space and access to the FB on offer to reduce competition; however, this will not prevent competition entirely.

## 7.6 Milk response and substitution rate

The predicted increase of DMI and milk production in response to increased HA has been reported previously (Dalley et al., 1999; Dillon et al., 2002; Auldist et al., 2013). Increased HA and rate of supplementation are also known to increase the substitution of herbage for a supplement (Penno et al., 2006). Thus, MINDY predicted the milk response to supplement (kg milk/kg DM of FB) would also decline with FBA, reflecting the increased substitution of herbage for FB, and the negative correlation with substitution rate (Bargo et al., 2002). Conversely, restricted pasture allocation is also known to increase the milk response to supplement due to greater utilisation and nutrient use efficiency, which support our results (Phillips, 1988; Penno et al., 2006). Increasing the HA from 25 to 40 kg DM/cow/d reduced the milk response to supplement from 1.36 to 0.96 kg milk/kg concentrate (Stockdale et al., 1990; Bargo et al., 2002). However, MINDY's predicted response to FB supplement was comparatively lower, as the maximum response achieved was ~1 kg milk/kg DM FB when 2FB was fed in the afternoon and supplemented a restricted HA (18HA fed AM+PM). Moderate milk responses (0.93 kg milk/kg DM concentrate) have also been reported when silage is supplemented with starch-rich grains such as barley. However, the supplementation rate (8.2 kg/cow/day) was considerably greater than those used by the model (Crosse and Gleeson, 1986). The low milk production response to FB may also be a consequence of the low DM of the bulb, as low DM forages increase ruminal fill compared with high DM, starch-rich, cereal grains (Phillips, 1988; Stockdale et al., 1990).

MINDY predicted a substantial difference in milk response between low HA and high HA. The negative milk response simulated by feeding 48 kg DM of herbage/cow per day, reflect increased substitution of herbage for FB, which is consistent with previous research (Waghorn et al., 2019). There appears to be little advantage to postpartum milk production or energy balance when the structural carbohydrates in herbage are replaced with non-structural carbohydrates found in cereal grains (Roche et al., 2006), sucrose (Penner and Oba, 2009) or fodder beet bulbs (Waghorn et al., 2019). This lack of early lactation milk response probably reflects the negative energy balance experienced by all modern dairy cows *postpartum* (Roche, 2006). However, as there are a limited number of studies which have assessed the milk response (kg milk/kg DM supplement) to fresh FB, further research is still needed.

Sucrose, the predominant sugar source in FB (Clark et al., 1987), is reported to improve DMI (Chamberlain et al., 1993; Broderick and Radloff, 2004; Penner and Oba, 2009). Supplementation of a diet consisting of herbage and maize silage with low amounts of liquid molasses increased milk production below an upper limit of 9% of daily intake (Broderick and Radloff, 2004). The reported improvement of ruminal pH when sucrose replaces starch increases DMI (Chamberlain et al., 1993; Penner and Oba, 2009). However, this does not always translate to greater milk production (Broderick and Radloff, 2004; Penner and Oba, 2009). Replacing starch with sugar reduces glucogenic precursors' supply, which may limit milk response from a sugar-rich supplement feed (Evans and Messerschmidt, 2017). Further limitations to milk production may also be a consequence of lower gross energy (GE) as Waghorn et al. (2019) reported GE values for FB bulb was less than pasture (16.3 versus 18.7 kJ/ g DM).

### 7.6.1 Ruminal fermentation

Except for lower concentration of all VFA and a slight increase of propionate associated with increasing FBA, rumen fermentation profiles were similar across diets. Changes of fermentation profiles in response to sucrose or FB supplementation are variable, with some accounts of increased proportions of butyric acid when sucrose replaces starch in the diet (Khalili and Huhtanen, 1991; Chamberlain et al., 1993), or FB supplements herbage (Eriksson et al., 2004; Waghorn et al., 2019; Pacheco et al., 2020). In contrast, Pacheco et al. (2020) found that propionate concentrations increased in response to FB supplementation. These responses reflect the altered chemical composition of the diet as the formation of propionate is increased with glucogenic precursors such as starch, while butyrate reflects greater dietary sugar content, due to FB (Oba, 2011). While FB reduced the content of fibre, increased proportions of butyrate may be due to hydrogen concentrations which alter fermentation thermodynamics (Hegarty and Gerdes, 1999). However, further research is required to evaluate how FB may affect the hydrogen dynamics and fermentation end-products *in vivo.*

### 7.6.2 Environmental pollutants

Dietary supply of crude protein was below the recommended 17-19% DM when 7FB diets were fed (Satter and Roffler, 1975). Feeding MINDY all FB allocations A+PS improved herbage intake and subsequently, the dietary protein supply. Nitrogen intake was not altered by the time of herbage allocation, and rumen ammonia concentrations were similar to previous

empirical studies (Trevaskis et al., 2004; Gregorini, 2012). MINDY predicted urinary N excretion would decrease with increased FBA although, the empirical evidence suggests that FB may have minimal impact on urinary N when fed 25:75 with herbage (Dalley et al., 2019). The increase of enteric methane emission with FBA suggest FB may cause pollution as Pareto front analysis failed to identify diets which would realistically improve both urinary N and $CH_4$ intensity, without causing SARA.

The twice-daily feeding regimen of FB not only increased ruminal pH but also reduced ammonia concentrations compared with once-daily supplementation. Increasing meal frequency of cereal grains which are rich in rumen degradable starch, slow the rate of carbohydrate degradation which may help stabilise ruminal pH and increase the microbial utilisation of ammonia (Stockdale et al., 1987). While the low N content of FB may reduce urinary N excretion when fed in large quantities, the potential to synchronise soluble protein with WSC may be limited under pastoral conditions. The considerable temporal, spatial and seasonal variation of nutrients across herbage swards and therefore, the timing of their availability in the rumen may prevent any benefits from nutrient synchrony in pastoral grazing systems (Hall and Huntington, 2008). While our results imply tangible changes to rumen digestion and pH even at low FBA, experimental work is required to explore the effect of rumen degradable protein and carbohydrate supply when supplementing grazed pasture with FB, if FB is used to reduce N excretion.

## 7.7 Conclusion

Sub-acute ruminal acidosis is predicted by MINDY when FB intake exceeded 27% of daily DMI. The results from this study suggest cows may be more susceptible to acidosis when FB is fed in early lactation and will experience a greater discomfort relative to the amount of FB fed. However, feeding FB twice-daily improved intake, milk production and rumen pH compared with once-daily feeding. The Pareto front analysis of model predictions suggested that milk production and total discomfort may be improved when small amounts of FB (1 kg DM) are fed twice daily, alongside 48HA. However, MINDY does not consider the complex feeding behaviours caused by competition and which may increase the variation of FB intake and risk of SARA, when translated at the herd scale. Twice daily feeding of FB will also increase labour and machinery costs which in addition to the extra cost required to harvest FB in spring, and the low milk response to supplement, suggest that FB may not be a cost-effective supplement under pastoral grazing conditions.

# Chapter 8

# Fodder beet to support early and late milk production from pasture, is it worth the risk?

Published in the Journal of Agricultural Systems (November 2020)

## 8.1 Abstract

High yielding crops such as maize (*Zea mays L.*) and fodder beet (FB; *Beta vulgaris L*), are commonly used to extend lactation and increase animal productivity from pastoral dairy systems. Financial modelling to compare costs and benefits of different crops is useful for decision making, but such modelling often fails to account for potential animal health risks associated with feeding supplements. A multi-component, whole-farm modelling approach was used to predict milk solids (MS, milk fat + protein) production and the economic farm surplus (EFS: operating surplus – adjustments) between 2016 to 2018 for an irrigated farm in Canterbury (South Island) and a non-irrigated farm in the Waikato (North Island), of New Zealand. The dairy business's financial risk was measured using the ratio between mean return on assets (ROA) minus an assumed 5% risk-free ROA, and the standard deviation of ROA was calculated from 300 combinations of climate, milk, and feed price, land appreciation, and interest rate. Four scenarios of autumn and spring supplementation of pasture were considered at each geographical location; imported maize silage (Base), a crop of maize silage grown on the milking platform (MSC; area used to produce milk), a crop of FB grown on the milking platform (FBC), and a FB crop with an outbreak of acute (1% stock fatality) and subacute ruminal acidosis (5% decline of feed intake) across the entire herd (FBAC). The MSC scenario improved EFS by 5.8% compared with Base in both the irrigated and the dryland system. The predicted response to MSC reflected greater milk production, lower feed expenses, and shorter crop rotation, compared with either Base, FBC, or FBAC. While FBC increased EFS by 4.8% compared with Base under irrigation, EFS was similar to Base under dryland conditions ($2,711 and $2,759/ha, respectively). The limited advantage of growing FB under dryland conditions reflect reduced herbage supply due to the extended crop duration of FB compared with maize silage. Model predictions suggest that FBAC will increase the financial risk by reducing milk production and EFS by 6.5% (irrigated) and 7.1% (dryland) compared with Base. In the absence of any adverse health risks, farm performance from the

FBC scenario was comparable to that of MSC under irrigated conditions. However, in dryland conditions, and when the potential economic cost of acute and sub-acute ruminal acidosis is considered, there is little advantage to growing FB on the milking platform.

## 8.2 Introduction

Pastoral dairy production systems in NZ are heavily reliant on traditional perennial ryegrass (*Lolium perenne*; PRG) and white clover (*Trifolium repens*; WC) swards to meet the energy demands of dairy cattle. While PRG x WC swards are an inexpensive and simple combination of pasture species to graze, additional inputs (imported feed or forage crops) are needed to meet animal requirements between late autumn and spring, with additional feed inputs or irrigation required in areas that are prone to drought during summer months (Clark et al., 2001; Neal et al., 2009). The heavy reliance on PRG x WC herbage for milk production and the deregulated nature of the NZ dairy industry expose NZ dairy producers to several business risks which include environmental, input, and output price, climate, biological, and production risks (Chapman et al., 2007). The combination of high relative costs for feed supplements, extreme market fluctuations and homogeneity of income source (milk sales represent more than 90% of net income), constrain operating profit and resilience of New Zealand dairy businesses (McCall and Clark, 1999; Neal and Roche, 2020). System intensity of NZ dairy systems are defined by the amount of feed that is imported onto the milking platform ranging between 0-5; System 1 - 0% of feed imported, System 2 - 4-14% of feed imported, System 3 - 10-20% of total feed imported, System 4 - 20-30% of feed imported, and System 5 - > 30% of feed imported. Operating profit is maximised by either constraining costs and importing some feed to mitigate the seasonality of herbage growth (System 3) or by increasing stocking intensity and importing more feed to maximise production (System 5) (Shadbolt et al., 2017). The investment of capital for infrastructure, feeding systems, stock, and land that are needed to attain high levels of milk production per ha can dilute the return on equity (ROE) from a level 5 system, and return on total dairy assets (ROA) are comparable with a System 3 intensity, despite the lower output per ha (Shadbolt, 2012). Comparison of either system's competitive strategy suggests that System 3 farms are more financially resilient long-term in both mitigating downside and capturing upside risk (Doole and Romera, 2015; Wales and Kolver, 2017; Neal and Roche, 2020). However, despite the improvement of forage yields using irrigation, plant breeding and grazing management, there is currently limited capacity to improve production and profit from medium intensity systems.

One option for improving the performance of low to medium intensity systems is integrating crop sequences to improve the 'feed base' by incorporating forage crops that increase DM tonnage and feed efficiency (Ho et al., 2013; Wales and Kolver, 2017). Fodder beet has been adopted as a winter forage, due to high yields (>20 t DM/ha) (Chakwizira et al., 2016), of a sugar-dense and highly utilisable bulb which is sown in spring (October - November), grazed during late lactation and winter, or harvested and stored for ~ 3 months above ground and fed as an early lactation source of water-soluble carbohydrate (WSC) in spring. However, the integration of complementary forages to circumvent seasonal herbage deficits can reduce profit and increase risk exposure, particularly during periods of climatic and economic adversity (Fariña et al., 2011; Rawnsley et al., 2013; Doole and Romera, 2015). The extended growing season (> 200 days) and rotation length of FB (> 12 months) increase the opportunity cost compared with alternative forage crops such as maize grown for silage, which has a shorter growing season (~150 days) and is reintroduced to the grazing rotation within six months (Fausett et al., 2015). Variation of the cost of inputs and outputs will also change the point of profit maximisation across different locations in NZ. Thus, highlighting the need to assess the risk of different combinations and locations of crop inclusion.

Fodder beet represents an additional risk to animal welfare because of its potential to cause acute and subacute ruminal acidosis (SARA). The bulb of FB is rich in WSC (principally sucrose), which is readily fermented and can cause volatile fatty acids (VFA) to accumulate and reduce ruminal pH (Owens et al., 1998). Sub-optimal ruminal pH prevents microbial degradation of structural carbohydrates, e.g. cellulose and hemicellulose, and cause anorexia, reduces rumination and the secretion of saliva which contains pH neutralising buffers (Nocek, 1997; Kleen et al., 2003). Poor feeding management of readily fermentable feeds may increase the permeability of bacterial endotoxin across tight junctions and thus impair the structural integrity of the rumen lining, limiting VFA absorption, animal production, and welfare long-term (Owens et al., 1998; Zebeli and Metzler-Zebeli, 2012b). Translocation of bacterial endotoxin into the circulation may cause systemic inflammation, increasing the incidence of laminitis and suppressing immune function (Nocek, 1997). In SARA, these symptoms are transient, pH is restored without intervention, and symptoms often pass undetected. In contrast, acute rumen acidosis (RA) is characterised by proliferation of lactic acid producers such as *Streptococcus bovis*, in the rumen, which further prevents ruminal pH from stabilising

and without intervention, can lead to metabolic acidosis that is fatal if not diagnosed and treated (Owens et al., 1998).

Substantial stock fatalities due to mismanagement of FB have been reported anecdotally (Marshall, 2011; Wallace, 2016). While careful transitioning to FB is known to reduce the incidence of stock fatality, industry reports suggest fodder beet related fatalities still exist (Beef+LambNZ, 2017; DairyNZ, 2017). However, such reports do not quantify the prevalence of RA and SARA across the herd. While the incidence of RA in experimental work is typically low under controlled conditions, Waghorn et al. (2018) reported RA in five of eight non-lactating cows following 14 days of transition to a FB (86%) and straw (14%) diet despite previous evidence that non-lactating cattle can be fed *ad libitum* FB without any immediate adverse health effects (Prendergast and Gibbs, 2015). It is generally well understood that profit is the risk-reward, yet NZ dairy producers cannot currently make informed decisions regarding the risk associated with feeding FB. Therefore, a whole-farm systems analysis of the economic impact of FB is needed to better quantify the potential financial losses due to RA in FB feeding systems.

The purpose of this modelling exercise was to compare the profit and risk of irrigated (Canterbury) and dryland (Waikato) dairy systems in which maize silage (which has minimal risk to animal health) is imported, maize silage is grown on the milking area, FB is grown on the milking area, or FB is grown and causes RA and SARA.

## 8.3 Methods

### 8.3.1 Model Description

The DairyNZ Whole Farm Model (WFM) predicts outputs from individual paddocks and animals in pastoral dairy systems (Beukes et al., 2005a; Beukes et al., 2008). The model exists as a framework linking component models for pasture (climate-driven), animals and crops with management decisions and climate. The animal component model, MOLLY is a mechanistic and dynamic model of a grazing dairy cow which stimulates metabolism, digestion, growth and lactation from the feed given (Baldwin, 1995) and is influenced by the chemical components of the diet and photoperiod (Beukes et al., 2005). Daily data for weather consist of comparative rainfall and solar radiation of NZ, which drive the component model for pasture (McCall and Bishop-Hurley, 2003). Animal and paddock production outputs from the WFM are then used in an economic component model to predict net income, variable

expenses (e.g. feed, fertiliser and grazing costs), farm working expenses (FWE), operating profit (net income – FWE), economic farm surplus (EFS; operating profit – adjustments) and ROA across climate years. The risk of each scenario was evaluated across climate years using a Monte-Carlo technique to calculate the Sharpe ratio. The Sharpe ratio indicates the excess ROA per unit of risk in response to climate variation, market volatility (feed and milk price) and appreciation of capital assets (Neal et al., 2005). Beukes et al. (2008) provide a detailed description of the WFM.

### 8.3.2 Scenarios

Economic and production outputs were predicted using industry averages for input costs (DairyNZ, 2018a) over two seasons from the 1st of June 2016 to the 31st of May 2018. Four farm systems were designed. Base, a PRG x WC system with imported maize silage. MSC, platform-grown maize silage with imported and platform-grown ryegrass silage. FBC, platform-grown FB for autumn and spring feeding with imported and platform-grown ryegrass silage. FBAC, the FBC scenario with an outbreak of acidosis during FB feeding. Each option was assessed across two regions in NZ, an irrigated system in Canterbury, South Island NZ (System 3-4: between 20-30% of feed imported) and a dryland system in the Waikato, North Island NZ (System 3; < 20% of feed imported), which has a warmer climate and greater rainfall (600 versus 1200 mm/yr.). The Canterbury scenarios were based on the Lincoln University Demonstration Farm (LUDF: 43°38'S, 172°27'E) with an average stocking rate of 3.2 cows/ha and irrigation (up to 600 mm) applied between September and April. Cows modelled were a Friesian x Jersey (F8J8) Kiwi cross weighing 495 ± 82.9 kg (mean ± sd). The Waikato scenarios were based on the DairyNZ Scott Farm (-37.7˚46'S, 175˚19'E), a dryland system with a stocking rate of 3.0 cows/ha, also Friesian x Jersey (F8J8) and weighed 505 ± 83.3 kg liveweight. The acidosis scenario was designed to calculate the economic effect of a flat rate 1% death from acute acidosis and 5% reduction of intake across the entire herd over each FB feeding period in autumn and spring. Incidence rates were based on a combination of anecdotal reports, and scientific research on the animal response to SARA (Khafipour et al., 2009a; Khafipour et al., 2009b; Waghorn et al., 2018; Waghorn et al., 2019). Estimated DMI is reported to decline 10% across reports of individual animals experiencing SARA; however, a 5% decline of DMI across the entire herd was assumed in the current study, due to a paucity of empirical data. Incidence of SARA was repeated at each FB transitioning event to represent that incidence of acidosis occurring in individuals across the herd even when using best practice management (Pacheco

et al., 2020). Scenarios simulated outputs daily over two years which began with pregnant non-lactating cows on the 1st of June 2016 and finished at the end of lactation on the 31st of May 2018.

### 8.3.3 Cropping and re-grassing policies

Both maize and FB crops were incorporated into a 10-year re-grassing policy to renew PRG x WC herbage. Following the 9$^{th}$ year of PRG x WC, 7% (FB) and 8% (maize silage) of the farm area was removed from the grazing rotation, cultivated and FB was sown ~ the 1st of September (Waikato) and ~ the 1st of October (Canterbury) for grazing on second and 16$^{th}$ March allowing approximately 212 and 197 days of growth for each scenario. Fodder beet was grazed up to 5 kg DM/cow/day until the end of lactation (May). Residual FB following autumn grazing remained in the ground over winter and was harvested and fed to support early lactation in spring. Maize was sown on the 25th of September (Waikato) and the 9th of October (Canterbury), harvested for silage on the 10th of March (Waikato) and the 25th of March (Canterbury), allowing for a growing season of ~152 days for each region. PRG x WC herbage was re-sown approximately three weeks following the harvest of each crop, and return of the paddock into the grazing rotation was dependant on climate and growth of the pasture. Therefore, paddocks sown in FB for either autumn or spring supplementation of herbage were returned to PRG x WC sward at the same time. The average yield of FB was 21 t DM/ha for Waikato and 23 t DM/ha for Canterbury. While the WFM model can predict the expected crop yield of maize silage, it has not yet been developed to predict crop yields of FB based on weather and management data, and DM yields of FB and maize silage crops were user-defined. Information describing FB yields in the Waikato was limited compared with Canterbury; thus rain-fed FB yields in the Waikato were assumed to be less than from irrigated crops in Canterbury (Chakwizira et al., 2014). Maize silage yields were 20 t DM/ha for Waikato and 19 t DM/ha for Canterbury scenarios. Yield assumptions for maize silage were based on a combination of AIMI reports 2013-2019 (Arable Industry Marketing Initiative) and data comparing regional differences of maize silage yield (Morris et al., 2016), and reflecting the cooler temperatures and shorter frost-free season which is experienced in Canterbury (Moot et al., 2007; Fletcher et al., 2011). Maize silage crop was grown on 8.5% of the milking area, while FB represented 7% of the milking area due to the greater yield of FB.

### 8.3.4 Management decisions

Daily allocation of PRG x WC across all scenarios was dependant on the available herbage mass and growth rate and was defined using the management decision rules outlined in Macdonald and Penno (1998). Rotation length across each season and location were defined based on data from previous seasons for each farm. Decisions to conserve herbage or graze were made according to the herbage growth rate and animal energy requirements during the period the paddock would be closed. Feed management of FB and maize silage were user-defined, 5 kg DM/cow per day of maize silage was fed from the 1st of March until dry off while up to 5 kg DM of FB was available for grazing from approximately the 2$^{nd}$ (Waikato) and the 20th of March (Canterbury) until dry off. Following autumn grazing, residual FB bulb was harvested and 4 kg DM/cow/day was fed between the 17th of August and the 30th of November for Waikato scenarios and between the 1st of September and the 15th of December in Canterbury. The chemical compositions of feeds were sourced from a database within the WFM and are presented in Table 8.1.

Across all simulations, heifer calves were kept as replacements and grazed off-farm until their first calving as rising two-year-olds. Each season 20% of the herd was replaced with rising-two-year-old heifers. If the percentage of empty cows was < 20 % of the original herd size, culling decisions were based on milk production, liveweight and age. Animals were dried-off due to body condition score (BCS) or low milk yield (< 10 L on average over the last seven days) between the 15th of April and the 15th of May (Waikato) and the 1st of March to the 20th of May (Canterbury). In the Waikato, cows often remain on the platform over winter. However, all cows from all scenarios were sent off farm over winter to simplify profit comparisons. In the Waikato, cows were grazed off-farm from the 25th of May to the 15th of July, while in Canterbury cows were grazed off-farm from the 21st of May to the 15th of July. During winter, the diet fed to cows is not defined within the model when cows are sent to an off-farm commercial 'grazier'. Therefore, each scenario received the same winter diet needed to achieve a target 5.0 BCS prepartum.

### 8.3.5 Economic input

The WFM predicted economic farm surplus from production and economic inputs from one year (the 1st of June 2016 to the 31st of May 2017). Economic inputs were derived from farm surveys conducted by DairyNZ Ltd for the 2016-17 season. Across the scenarios, variable costs

were altered due to supplement type and price. Cost of imported maize silage was based on actual data from four seasons in Waikato (28 ± 2.4 c/kg DM) and Canterbury (30 ± 2.2 c/kg DM). Across both regions, the growing cost of FB was estimated at $2,500/ha, while the costs of harvesting and handling were assumed as 6c/kg DM of FB bulb. Costs associated with feeding out supplement were on average, $45/t DM across all scenarios and types of supplement. When grazed in situ, the utilisation of FB was 90% based on a previous study (Saldias and Gibbs, 2016). The utilisation of FB once harvested has not been investigated, and 75% was assumed (accounting for biomass lost during harvesting, storage and feeding out processes), this rate was also used for maize silage.

### 8.3.6 Output

Outputs requested from the model included daily herbage growth of PRG x WC (kg DM/ha/day) and average pasture cover of the farm (kg DM/ha), herbage grazed and harvested for silage, the metabolisable energy content of the feed (MJ ME/kg DM), nitrogen applied as effluent and urea, irrigation (mm), soil moisture (%), daily maximum and minimum temperatures (℃). Variables of animal production included daily liveweight (kg), milk production (kg/cow/d), yields of milk constituents (milk fat, protein, MS, and lactose; kg/cow/day), nitrogen (N) intake, N excretion (kg N/cow/day), daily (g) and seasonal (kg) methane emission per cow and herd. Net income was predicted based on the Fonterra milk price schedule for 2016-17 multiplied by MS production in addition to livestock income.

### 8.3.7 Risk analyses

The financial risk was defined using the Sharpe ratio (Neal et al., 2005; Neal and Cooper, 2016) in which production and subsequent return on asset (ROA) from each scenario were predicted from 100 combinations of milk, supplement price, land appreciation and interest rate over three production years (2014-16, 2015-17, and 2016-18), resulting in 300 combinations for each scenario. The average milk price was user-defined (6.00/kg MS) and ranged from $10.50 to $3.48/kg MS, the range of milk price across each production year was maintained across each scenario. The mean cost of supplement for pasture silage was linked to weather and correlated with milk price, i.e. the cost of supplement declined when milk price was low and increased during seasons of low rainfall (Shadbolt et al., 2017). The Sharpe ratio was then calculated using the average ROA minus an assumed 5% risk-free ROA rate (assuming perfect market conditions), divided by the standard deviation of ROA (Neal et al., 2005).

$$Sharpe\ Ratio = \frac{(ROA - 5\%)}{SD\ of\ ROA} \quad (8.1)$$

The Sharpe ratio reflects the probability distribution of mean return on asset where a high ratio indicates a superior choice that improves ROA and reduces risk (Chapman et al., 2007). While growth and yield of pasture herbage were driven by weather, the effect of crop yield of maize silage and FB on profit and risk was determined independently by running each cropping scenario (MSC, FBC and FBAC) with variations of yields (17-31 t DM/ha in 2 t DM/ha increments) at both geographical locations.

### 8.3.8 Data analysis

Daily weather and annual climate, and economic and production outputs were averaged across months and season (between the 1st of June and the 31st of May). The herd in each system represented the experimental unit of interest and did not present any variation, and only absolute differences are reported.

## 8.4 Results

### 8.4.1 Weather

Monthly rainfall accumulations are displayed in Figure 8.1. Rainfall in Waikato totalled 1,447 and 1,465 mm across the 2016-17 and 2017-18 seasons respectively. While total rainfall accumulations were similar between the seasons in the Waikato, approximately 270 mm of rain fell in January in 2018 which was considerably greater than the 50 mm which fell in January 2017 (Figure 8.1). The high rainfall experienced in January increased the average soil moisture by 17.1 mm and reduced ryegrass herbage utilisation by 10%. In Canterbury, rainfall increased 346 mm in the second season, reducing the need for irrigation and maintaining soil moisture content between 70-80% of field capacity

Table 8.1. Chemical composition of whole fodder beet (FB) and FB bulb, maize silage, pasture silage and a perennial ryegrass/white clover herbage grown with (Canterbury) or without (Waikato) irrigation

| Plant | DM[1] % | ME[1] MJ/kg DM | CP[1] % | Fat% | Starch % | WSC[1] % | NDF[1] % | ADF[1] % |
|---|---|---|---|---|---|---|---|---|
| FB bulb | 16.3 | 11.9 | 7.00 | 5.15 | 1.03 | 72.0 | 10.7 | 5.70 |
| FB grazed | 17.1 | 12.0 | 5.61 | 4.10 | 6.15 | 59.5 | 13.1 | 8.56 |
| Maize silage | 33.0 | 10.3 | 9.30 | 3.50 | 20.6 | 4.6 | 48.6 | 29.6 |
| Pasture Silage | 25.0 | 10.6 | 22.4 | 4.00 | 5.72 | 16.7 | 34.5 | 27.5 |
| Ryegrass/clover Canterbury | 20.5 | 10.4 | 22.9 | 4.09 | 4.35 | 15.5 | 39.9 | 27.8 |
| Ryegrass/clover Waikato | 16.4 | 10.1 | 22.3 | 4.08 | 1.61 | 11.0 | 49.0 | 32.2 |

[1]DM:dry matter, ME: metabolisable energy, CP: crude protein. WSC: water soluble carbohydrate, NDF: Neutral detergent fibre, ADF: Acid detergent fibre

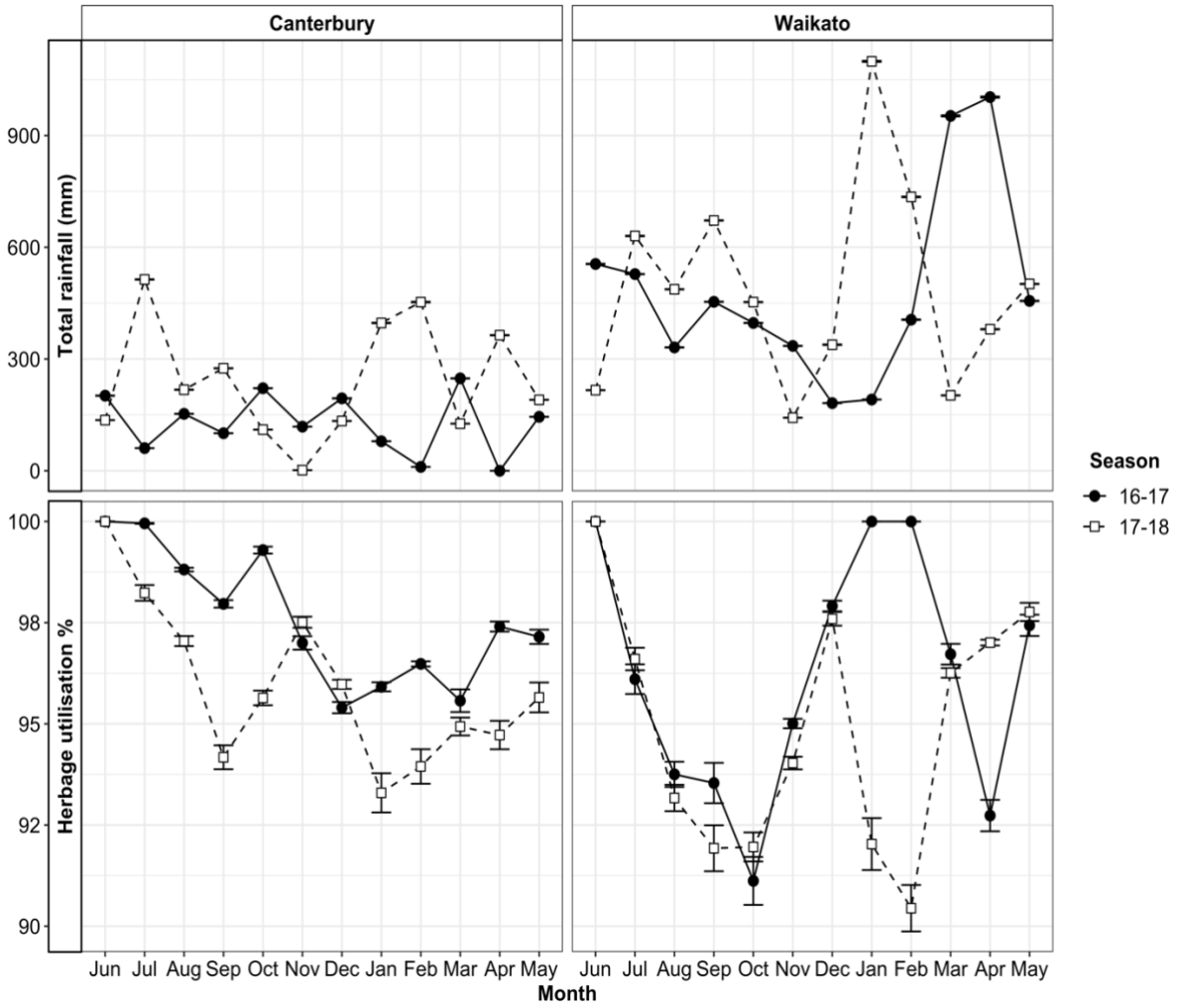


Figure 8.1. Total monthly rainfall (mm) and herbage utilisation (%) in Canterbury and Waikato from June 2016 to May 2017 and Jun 2017 to May 2018

Table 8.2. Stocking rate (SR), predicted seasonal milk solids sold (MS) net income, feed and grazing expenses, total farm working expenses (FWE), economic farm surplus (EFS; operating profit-adjustments) and return on total assets (ROA), for pastoral systems in Canterbury (irrigated) and the Waikato (non-irrigated).

| Production | Canterbury | | | | Waikato | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Base[1] | MSC[2] | FBC[3] | FBAC[4] | Base | MSC | FBC | FBAC | SEM[5] |
| Farm area (ha) | 160 | 160 | 160 | 160 | 140 | 140 | 140 | 140 | |
| Cows | 515 | 515 | 515 | 515 | 420 | 420 | 420 | 420 | 18.6 |
| SR (cows/ha) | 3.22 | 3.22 | 3.22 | 3.22 | 3 | 3 | 3 | 3 | 0.04 |
| MS (kg) | 236,443 | 234,009 | 232,790 | 225,452 | 189,801 | 191,287 | 187,970 | 185,816 | 8,580 |
| **Income** | | | | | | | | | |
| Milk ($) | 1,465,453 | 1,447,418 | 1,438,445 | 1,392,464 | 1,174,429 | 1,181,663 | 1,163,249 | 1,150,716 | 53,062 |
| Stock ($) | 160,530 | 160,670 | 160,670 | 151,420 | 106,680 | 117,790 | 110,430 | 106,380 | 10,599 |
| Net ($) | 1,632,081 | 1,614,185 | 1,605,556 | 1,549,981 | 1,286,445 | 1,304,788 | 1,279,014 | 1,262,431 | 62,904 |
| MS (kg/ha) | 1,478 | 1,463 | 1,455 | 1,409 | 1,356 | 1,366 | 1,343 | 1,327 | 21.8 |
| Milk ($/ha) | 9,159 | 9,046 | 8,990 | 8,703 | 8,389 | 8,440 | 8,309 | 8,219 | 135 |
| Stock ($/ha) | 1,003 | 1,003 | 1,004 | 1,004 | 1,004 | 978 | 946 | 574 | 51.8 |
| Net ($/ha) | 10,201 | 10,089 | 10,035 | 9,687 | 9,189 | 9,320 | 9,136 | 9,017 | 178 |
| **Expenses** | | | | | | | | | |
| Feed ($) | 180,847 | 131,596 | 129,726 | 125,845 | 138,556 | 122,418 | 131,057 | 134,403 | 9,099 |
| Grazing ($) | 209,998 | 207,830 | 203,260 | 212,758 | 220,190 | 219,823 | 219,638 | 219,090 | 5,771 |
| FWE ($) | 990,705 | 947,143 | 929,511 | 927,276 | 774,093 | 770,129 | 771,425 | 773,733 | 37,183 |
| Feed ($/ha) | 1,130 | 822 | 811 | 787 | 990 | 874 | 936 | 960 | 60.4 |
| Grazing ($/ha) | 1,312 | 1,299 | 1,270 | 1,330 | 1,573 | 1,570 | 1,569 | 1,565 | 64.0 |
| FWE ($/ha) | 6,192 | 5,920 | 5,809 | 5,795 | 5,529 | 5,501 | 5,510 | 5,527 | 126 |
| **Surplus ($)** | | | | | | | | | |
| EFS($) | 484,711 | 512,948 | 508,936 | 453,274 | 386,328 | 410,228 | 379,513 | 358,810 | 23,455 |
| EFS ($/ha) | 3,029 | 3,206 | 3,181 | 2,833 | 2,759 | 2,930 | 2,711 | 2,563 | 97.6 |
| ROA (%) | 11.0 | 11.4 | 11.3 | 10.7 | 10.3 | 10.6 | 10.3 | 10.0 | 0.41 |
| ROA % | 12.0 | 12.5 | 12.3 | 11.4 | 11.7 | 11.8 | 11.6 | 11.4 | |
| SD of ROA | 4.56 | 4.27 | 4.23 | 4.24 | 4.20 | 4.10 | 4.06 | 3.96 | |
| Sharpe ratio | 1.52 | 1.76 | 1.72 | 1.51 | 1.60 | 1.67 | 1.62 | 1.62 | |

[1]Imported maize silage fed in autumn and spring, [2]Maize silage grown on the platform, [3]FB grown on the platform for autumn grazing and harvested bulb is fed-out during spring, [4] FBC with an outbreak of SARA, (5% reduction of total intake and 2% stock fatality) [5]SEM: Standard error of mean, [6]Ratio of ROA and SD.

### 8.4.2 Net income

Sales of milk solids provided the majority of income for all scenarios (90-91%). Sales of livestock averaged 10.3% of net income across the Canterbury scenarios and 9.0% of net income across the Waikato scenarios. Stocking rate across the two seasons averaged 2.9 cows/ha in the Waikato and 3.2 cows/ha in Canterbury (Table 8.2). Despite the 2% annual death rate (1% during each FB transitioning event) caused by acidosis from FB, the culling policy maintained the baseline stocking rate of FBAC scenarios by culling fewer cows in autumn. On average, the greater death rate of the FBAC scenarios reduced total livestock sales by 3.7% in the Waikato and 5.7% in Canterbury compared with the FBC scenarios. In the Waikato, MS production increased by 0.8% with MSC, compared with Base but declined 1.0% with FBC and 2.1% with FBAC (Table 8.2). In Canterbury, total MS production was 1.0% (MSC), 1.5% (FBC) and 4.6% (FBAC) lower than the Base scenario. Milk solids production per cow was similar between the two regions, although Canterbury Base produced 7 kg MS/year more than the Waikato Base scenario (Table 8.2). Across the Waikato scenarios, predicted net income increased 1.4% with MSC but declined 0.6% with FBC and 1.9% with FBAC, compared with the Base scenario. Across the Canterbury scenarios, the predicted net income declined 1.1% with MSC, 1.6 % with FBC and 5.0% with FBAC (Table 8.2).

### 8.4.3 Farm working expenses

Feed expenses (excluding off-farm grazing costs) accounted for 13 to 18% of total FWE and was greatest in the Canterbury Base scenario (18.2% of FWE) while feed cost of MSC, FBC and FBAC were 13.9, 14.0 and 13.6% of FWE, respectively. Compared with the Base scenario in the Waikato, feed costs declined 13.2% (MSC), 5.7% (FBC) and 3.1% (FBAC). Growing forge crops reduced feed costs by 27.3% (MSC), 28.3% (FBC) and 30.4% (FBAC), compared with Base (Table 8.2), in Canterbury. Farm working expenses were also reduced by growing forage crops on the milking platform and declined; 0.5% (MSC), 0.4% (FBC) and 0.1% (FBAC) in the Waikato and 4.4% (MSC), 6.2% (FBC) and 6.4% (FBAC) in Canterbury compared with Base scenarios. Farm working expenses averaged $4.09/kg for MSC across both locations. Growing FB on the platform increased FWE by 2 c/kg MS in the Waikato compared with the Base scenario, but FWE were 20 c/kg MS lower in Canterbury. Compared with the FBC scenario, FBAC increased FWE by 6 c/kg MS in the Waikato and by 12 c/kg MS in Canterbury.

Table 8.3. Predicted herbage cover and growth available for grazing or harvesting (as silage) per day and annually, mean metabolisable energy (ME) content of the total diet fed and application of nitrogen (N) fertiliser and animal effluent of four pasture based-systems in Canterbury (irrigated) and the Waikato (non-irrigated).

| | Canterbury | | | | Waikato | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Base[1] | MSC[2] | FBC[3] | FBAC[4] | Base | MSC | FBC | FBAC | SEM[5] |
| **Daily Herbage** | | | | | | | | | |
| Pre-grazing cover | 2505 | 2382 | 2242 | 2256 | 2331 | 2375 | 2146 | 2155 | 110 |
| Post-grazing cover | 1752 | 1719 | 1663 | 1691 | 1599 | 1799 | 1603 | 1626 | 125 |
| Herbage growth | 40.7 | 40.6 | 38.6 | 37.8 | 38.9 | 35.1 | 35.7 | 35.2 | 12.0 |
| Herbage grazed | 34.6 | 35.6 | 37.4 | 36.5 | 36.5 | 33.5 | 35.8 | 35.5 | 7.99 |
| Herbage harvested | 6.3 | 7.6 | 7.9 | 8.0 | 2.1 | 3.4 | 4.0 | 3.9 | 1.00 |
| **Annual t DM/ha** | | | | | | | | | |
| Herbage grown | 14.8 | 14.8 | 14.07 | 13.8 | 14.2 | 12.8 | 13.0 | 12.9 | 0.37 |
| Herbage grazed | 12.6 | 13.0 | 13.7 | 13.3 | 13.3 | 12.2 | 13.1 | 13.0 | 0.17 |
| Herbage harvested | 2.2 | 1.2 | 1.8 | 1.9 | 0.7 | 0.5 | 0.6 | 0.6 | 0.30 |
| ME (MJ/kg DM) | 9.8 | 9.7 | 9.9 | 9.9 | 9.8 | 9.7 | 9.8 | 9.8 | 0.11 |
| **Nitrogen (kg/ha)** | | | | | | | | | |
| Fertiliser | 160 | 165 | 148 | 148 | 110 | 107 | 101 | 101 | 10.1 |
| Effluent | 18.3 | 19.2 | 17.0 | 16.5 | 14.0 | 14.6 | 13.5 | 13.1 | 1.82 |
| Total N | 178 | 184 | 165 | 165 | 124 | 122 | 115 | 114 | 9.61 |

[1] Maize silage imported to mitigate autumn and spring pasture deficit [2] Maize silage grown on the platform, [3]FB grown on the platform for autumn grazing, harvested and fed to cows in spring, [4]Investigation of FBC with an outbreak of SARA, defined as 5% reduction of total intake and 2% stock fatality, [5]SEM: Standard error of the mean.

### 8.4.4 Economic farm surplus

Compared with Base in Canterbury, the predicted EFS was 5.8% greater with MSC and 5.0% greater with FBC, but 6.8% lower with FBAC (Table 8.2). For the Waikato scenarios, MSC increased the EFS above Base, FBC and FBAC, in respective order (Table 8.2). Compared with Base, EFS increased by 6.2% with MSC but declined 1.8% with FBC and 7.1% with FBAC. The EFS/ha for Base was greater in Canterbury than in the Waikato ($3,062 versus $2,741/ha) (Table 8.2). The average ROA also increased in Canterbury compared with the Waikato scenarios (11.1 versus 10.3 %). The MSC scenario increased ROA above the Base scenarios in both regions. While the FBC scenario increased ROA above the Base scenario in Canterbury, ROA declined when compared with the Base scenario in the Waikato. The FBAC scenario reduced ROA compared with all scenarios in both Canterbury and the Waikato (Table 8.2).

### 8.4.5 Climate and herbage outputs

The greater rainfall experienced over summer in the second season in Canterbury reduced the amount of maize and ryegrass silage needed to meet animal requirements in Canterbury, but reduced feed utilisation in Waikato (Figure 8.1). Annual PRG x WC growth (t DM/ha) was greater in Canterbury than in Waikato (14.4 versus 13.2 t DM/ha/yr.). Average pre-graze PRG x WC cover was reduced by crop inclusion compared with imported maize silage in both regions (Table 8.3; Figure 8.2). The utilisation of PRG x WC herbage was similar across scenarios and only differences between regions are displayed (Figure 8.1). The FBC scenario increased the average ME content of the diet between August to October and February to May compared with Base or MSC scenarios (Figure 8.2). Across each season, herbage growth rate (kg DM/ha) was similar between scenarios, although herbage growth was greater from October to January for the Base scenarios compared with the cropping scenarios. In Canterbury, the yield of PRG x WC of Base and MSC was greater than FBC and FBAC (Table 8.3). However, under dryland conditions in the Waikato, the yield of PRG x WC in FBC, and FBAC surpassed that of MSC (Table 8.3). In Canterbury, utilisation of PRG x WC herbage increased 8.0% with FBC, 5.3% with FBAC and 3.1% with MSC compared with the Base scenario. In Waikato, the utilisation of PRG x WC herbage declined 1.5% with FBC, 2.3% with FBAC, and 8.3% with MSC compared with Base (Table 8.3).

### 8.4.6 Animal outputs

Across both regions, the WFM predicted the greatest average liveweight for cows fed MSC while the FBC and FBAC scenarios both reduced liveweight, compared with Base (Figure 8.3). However, the differences were minimal when averaged across animals and season (Table 8.4). Scenario effects were also minimal for average herd age, BCS and daily milk yield, MS (Figure 8.4), milk fat, protein, lactose, fat to protein ratio, milk N, DM requirements, DMI and concentration of N in urine and faeces (Table 8.4). Grazed PRG x WC herbage represented a greater component of the diet of the MSC cows compared with all other scenarios, including Base, in which maize silage was imported. In the MSC scenario, dietary intake of maize silage declined while intake of pasture silage increased compared with Base (Figure 8.5). At both locations, FB represented a greater component of the diet per cow than maize silage in MSC (Figure 8.5) and reduced the percentage of grazed PRG x WC eaten by the FBC cows.

Table 8.4. Predicted cow liveweight (LWT), body condition score (BCS), daily milk production and composition, dry matter requirements (DMR) and dry matter intake (DMI), nitrogen (N) intake, urinary N excretion and enteric methane ($CH_4$) produced daily and annually per cow from four pasture-based systems in Canterbury (irrigated) and the Waikato (non-irrigated).

| | Canterbury | | | | Waikato | | | | SEM[5] |
|---|---|---|---|---|---|---|---|---|---|
| **Production** | Base[1] | MSC[2] | FBC[3] | FBAC[4] | Base | MSC | FBC | FBAC | |
| LWT (kg) | 468 | 470 | 465 | 464 | 484 | 490 | 480 | 476 | 9.7 |
| BCS[6] | 3.49 | 3.52 | 3.38 | 3.33 | 3.54 | 3.64 | 3.47 | 3.42 | 0.25 |
| Age | 4.8 | 4.8 | 4.8 | 4.8 | 5.1 | 5.1 | 5.0 | 5.1 | 0.26 |
| Milk (kg) | 18.5 | 18.5 | 18.3 | 18.0 | 18.1 | 18.4 | 18.1 | 17.9 | 2.12 |
| Milk solid (kg) | 1.78 | 1.76 | 1.75 | 1.71 | 1.72 | 1.75 | 1.72 | 1.69 | 0.168 |
| Milk fat (kg) | 1.03 | 1.01 | 1.01 | 0.98 | 0.98 | 1.01 | 0.99 | 0.96 | 0.107 |
| Milk protein (kg) | 0.75 | 0.75 | 0.74 | 0.73 | 0.74 | 0.75 | 0.74 | 0.73 | 0.062 |
| Lactose (kg) | 0.89 | 0.89 | 0.88 | 0.86 | 0.87 | 0.88 | 0.87 | 0.86 | 0.095 |
| Fat : protein | 1.35 | 1.34 | 1.34 | 1.32 | 1.32 | 1.33 | 1.32 | 1.31 | 0.043 |
| **Intake** | | | | | | | | | |
| DMR (kg DM/cow.$d^{-1}$) | 12.7 | 12.7 | 12.8 | 12.9 | 13.0 | 13.0 | 13.1 | 13.0 | 0.85 |
| DMI (kg DM/cow.$d^{-1}$) | 12.7 | 12.7 | 12.8 | 12.7 | 13.0 | 13.2 | 13.1 | 12.9 | 0.87 |
| N (kg DM/cow.$d^{-1}$) | 0.44 | 0.46 | 0.45 | 0.45 | 0.47 | 0.48 | 0.47 | 0.46 | 0.023 |
| **N excretion** | | | | | | | | | |
| Milk N (kg/cow.$d^{-1}$) | 0.12 | 0.12 | 0.12 | 0.11 | 0.12 | 0.12 | 0.12 | 0.11 | 0.041 |
| Urinary N (kg/cow.$d^{-1}$) | 0.26 | 0.28 | 0.27 | 0.27 | 0.28 | 0.29 | 0.28 | 0.27 | 0.014 |
| Faecal N (kg/cow.$d^{-1}$) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.007 |
| **$CH_4$ emission** | | | | | | | | | |
| $CH_4$ g/cow/day | 303 | 302 | 298 | 296 | 312 | 312 | 305 | 300 | 2.07 |
| $CH_4$ kg/cow/yr. | 110 | 109 | 109 | 108 | 113 | 114 | 111 | 110 | 0.73 |
| $CH_4$ t/yr. | 56.6 | 56.3 | 56.1 | 55.7 | 47.6 | 47.9 | 46.8 | 46.3 | 1.72 |

[1] Maize silage imported to mitigate autumn and spring pasture deficit, [2] Maize silage grown on the platform, [3] FB grown on the platform for autumn grazing, harvested and fed to cows in spring.[4]Investigation of FBC with an outbreak of SARA, defined as 5% reduction of total intake and 2% stock fatality, [5] SEM: Standard error of the mean, [6] BCS: scale 1:5, 1 = emaciated, 5 = obese

The predicted annual methane yields (t/yr.) were similar across all scenarios but were 2.7% greater in the Waikato compared with Canterbury. Total annual emissions of enteric methane for each scenario were also similar and ranged from 56.6 to 55.7 t CH4/yr in Canterbury and 47.9 to 46.3 t $CH_4$/yr in the Waikato (Table 8.4).

### 8.4.7 Risk

The average Sharpe ratio of the Canterbury scenarios was greater than that of the Waikato (1.71 versus 1.65). The MSC scenario increased the Sharpe ratio by 1.9% in Canterbury and 3.4% in the Waikato, compared with FBC, when crop yields ranged from 17 to 30 t DM/ha (Figure 8.6). However, the Sharpe ratio of FBC (FB crop: 23 t DM/ha) was similar to MSC (maize crop: 19 t DM/ha) in Canterbury despite the 17.4% increase of FB yield. Growing FB on the platform increased the Sharpe ratio by 1.3% compared with Base in the Waikato, while MSC

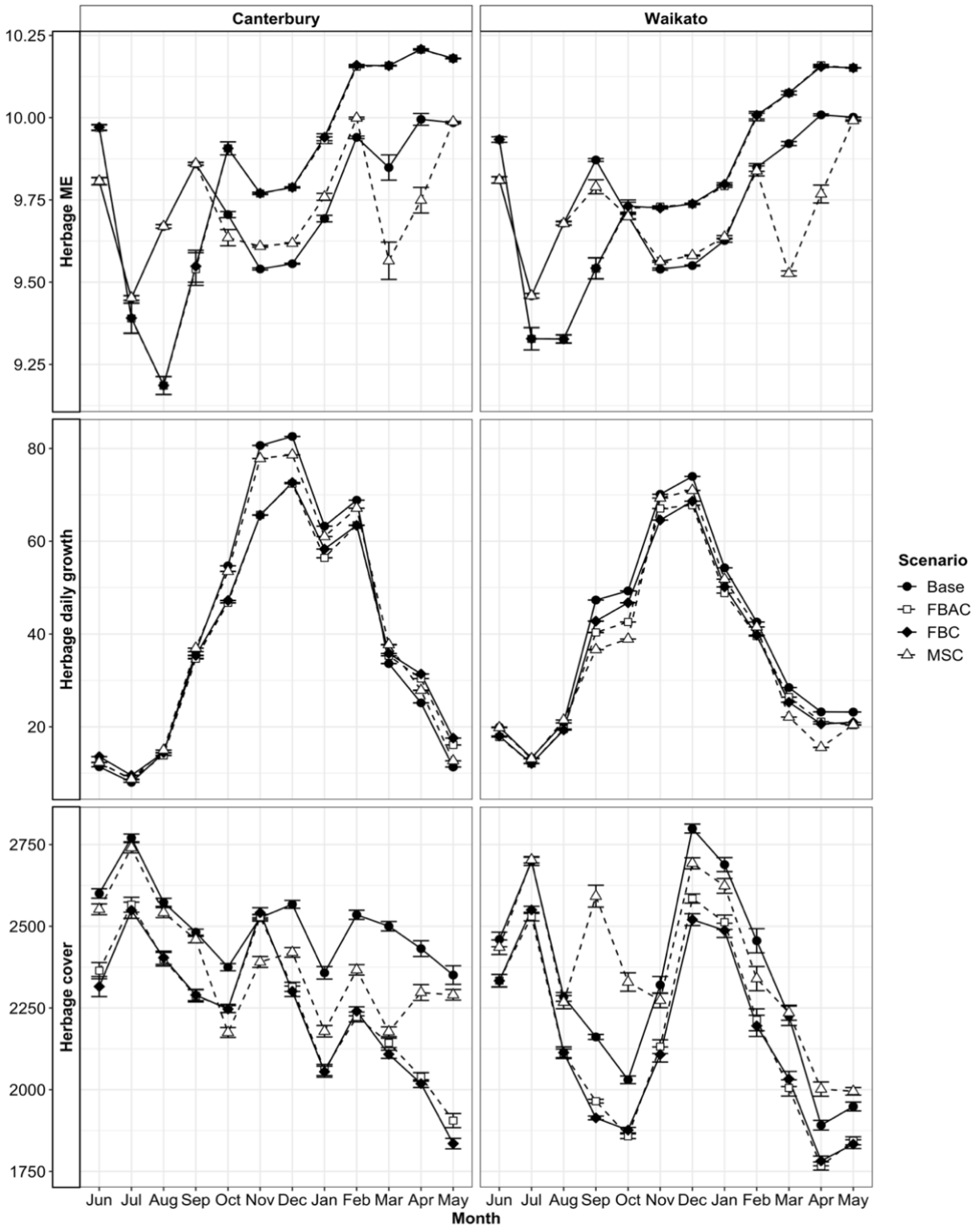


Figure 8.2. Average metabolisable energy (ME: MJ/kg DM) content, herbage growth (kg DM/ha) and cover (kg DM/ha) predicted for Canterbury and the Waikato, where maize silage is imported (Base) or either maize silage (MSC) or fodder beet (FBC) are grown on the platform. The FBAC scenario explores the effect of acute and sub-acute ruminal acidosis.

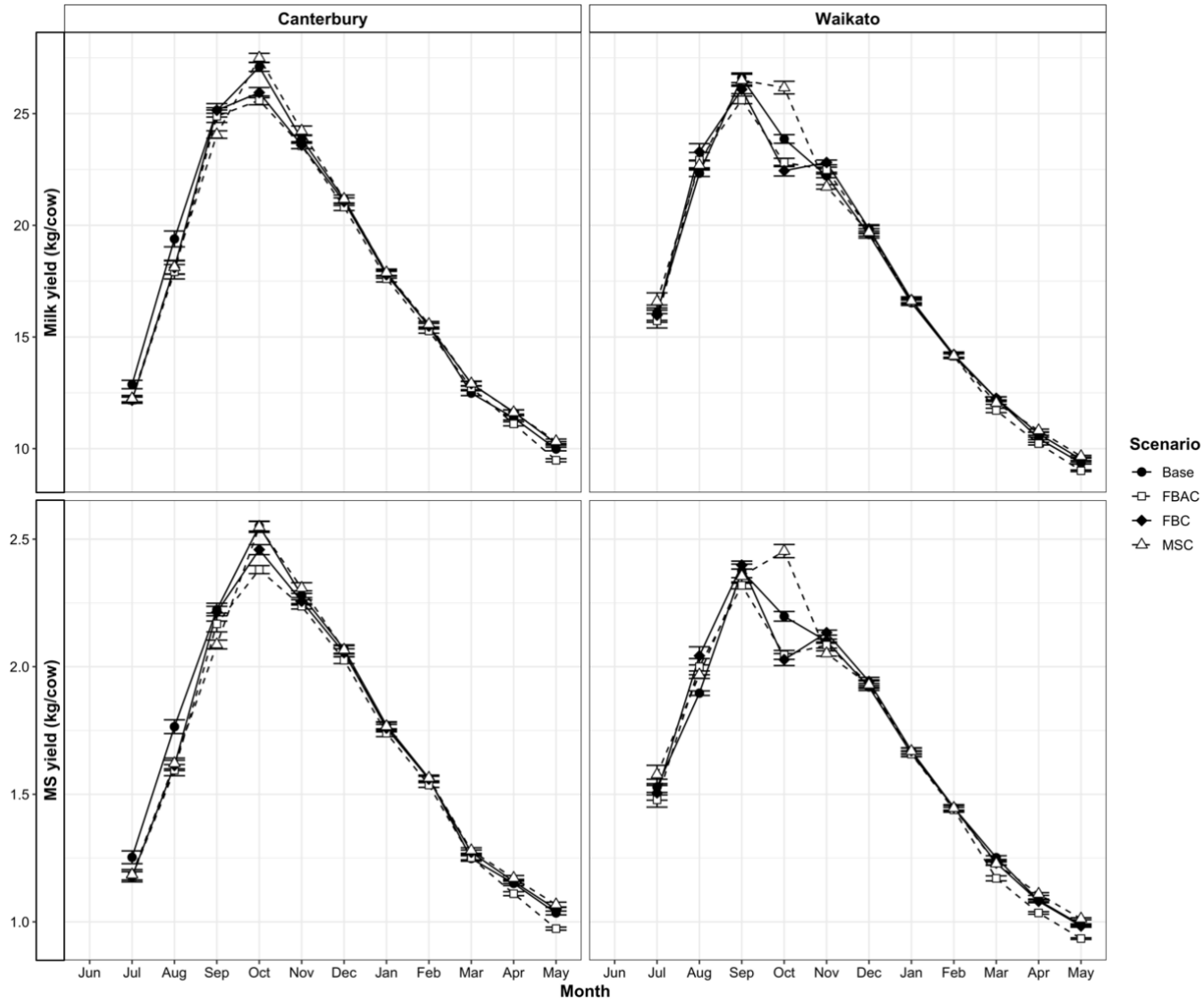


Figure 8.3. Predicted daily milk yield (kg/cow/day), and milk solids yield (kg MS/cow/day) of cows across two geographical locations (Canterbury and the Waikato). Scenarios are pasture-based systems where maize silage is imported (Base), grown on the platform (MSC) or fodder beet is grown on the platform (FBC). The effect of ruminal acidosis caused by FB is also investigated in the FBAC scenario

increased the Sharpe ratio by 4.4%. While ruminal acidosis simulated in the FBAC scenarios did not substantially change the Base value of Sharpe ratio (Table 8.2), ROA declined by 5% in Canterbury and by 2% in Waikato compared with the ROA of Base scenarios. The model predicted a positive relationship between the Sharpe ratio and crop yield, and the MSC scenarios produced greater Sharpe ratios than FBC at both locations (Figure 8.6).

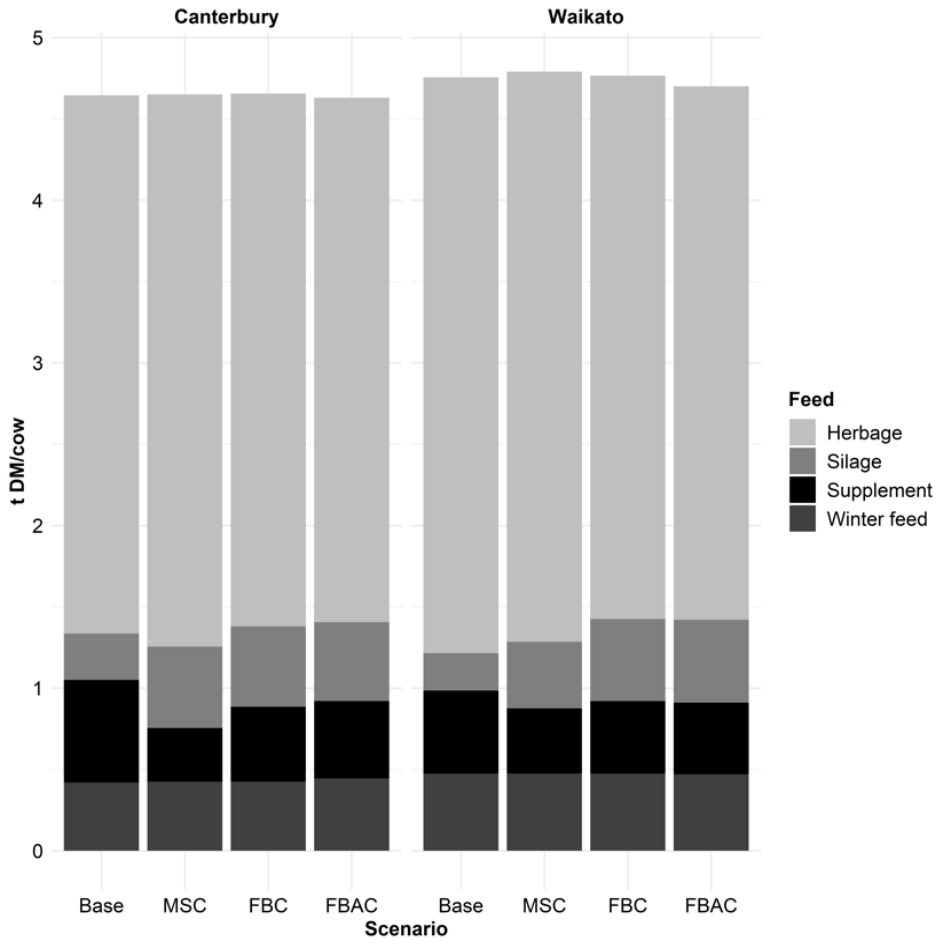


Figure 8.4. Total annual dry matter intake per cow (t DM/cow) of grazed herbage (herbage), herbage silage (silage), winter feed, and supplement of four scenarios of farm systems consisting of different types of supplement; imported maize silage (Base), maize silage grown on the platform (MSC), FB grown on the platform (FBC) and fodder beet with an outbreak of ruminal acidosis (FBAC). Feeding scenarios were repeated at two locations representing irrigated (Canterbury) and non-irrigated (the Waikato) dairy systems.

## 8.5 Discussion

### 8.5.1 Regional variation and system intensity

The predicted profitability of each scenario varied between the two regions due to a combination of stocking rate and input costs. For the 2016-17 season, predicted EFS ranged between $2,563 and $3,206/ha, which was within a similar range for the WFM as reported by Beukes et al. (2019). While MS production from the Canterbury Base scenario was greater than FBC or MSC, feed expenses also increased, and the EFS declined compared with other Canterbury scenarios, a well-established concept for NZ dairy farms (Macdonald et al., 2008; Doole, 2014; Doole and Romera, 2015). The stocking rate of Canterbury scenarios increased by 7% compared with the stocking rate of the Waikato scenarios, which was predicted to

increase milk sales by 8%. The predicted Sharpe ratio of Canterbury scenarios increased with forage crops compared with Base; MSC (17%) and FBC (13%). However, the differences between imported and cropping scenarios were modest under the dryland conditions experienced in the Waikato (4.2 and 1.0%, MSC and FBC, respectively), which may partially reflect the greater cost of maize silage in Canterbury compared with the Waikato ($300 versus $290/t DM).

Profit metrics such as EFS or ROA vary considerably from season to season and do not indicate long-term outcomes or resilience of the system; where resilience refers to the continuity of key components and relationships of the system through space and time (Cumming et al., 2005). While variable expenses are expected to fluctuate, they should not compromise the long term resilience, or the ability of the system to 'stretch' and the system should remain operational during unexpected events (Shadbolt et al., 2017). The Shape ratio indicates the resilience of the farm system by incorporating key biophysical relationships which underpin business risk (in response to altered farm practices) in a dynamic model of a pastoral dairy system. The system with the greatest Sharpe ratio will provide the best economic advantage and (assuming ROA meets the farmer's utility function or goals), will be the best option regardless of the farmer's desired level of risk aversion (Neal and Cooper, 2016). While growing either maize or FB on the milking platform did not substantially improve risk outcomes in the Waikato, the increased Sharpe ratios predicted in Canterbury suggest an advantage for growing forage crops on the milking platform when irrigation is available to maintain herbage growth. However, research at greater and lower crop inclusion levels is needed to further establish the potential for complementary forages to improve profit and risk outcomes of moderately intensive pastoral systems.

The point of profit maximum will affect how the system should respond to an altered operating environment. For example, when milk price is high, the value of grazed herbage increases. The increased land-value will increase the value of herbage or forage crops grown on the platform, and importing supplement feeds may become more profitable (Kolver et al., 2001). The comparison of Sharpe ratios suggests that crop inclusion does not increase the risk exposure compared with an imported supplement, which disagrees with previous research (Rawnsley et al., 2013). However, the model predictions suggest that the reduced reliance on imported feed when milk price is low, by growing forages on the milking platform, is advantageous to ROA and the Sharpe ratio. The model predictions further emphasise the need

to minimise feed expenses due to the deregulated economy of the NZ dairy industry which directly exposes NZ dairy farmers to milk prices which are variable and generally low (Macdonald et al., 2001). From the analysis of 12 years of Dairybase data (a NZ database of farm physical and financial information), three years recorded high milk price ( > 7.50/kg MS NZD) while six years recorded low milk prices (< $6.50/ kg MS NZD) (Neal and Roche, 2020). The Sharpe ratio improvement due to the inclusion of forage crops highlights the advantage of a resilient business structure that can withstand variations of input and output values (Shadbolt et al., 2017). Stochastic modelling has indicated that risk and farm profit are optimised when crop area is between 10-20% of the farm area (Neal et al., 2010). In the current study, the crop area was < 10% of the platform, reducing the risk of feed deficits that lead to expensive 'on the spot' purchase of imported feed. While the previous study in Australia indicates the inclusion of forage crops increase risk exposure (Neal et al., 2010; Ramsbottom et al., 2015; Romera and Doole, 2015), the reduced price differential between milk income and supplemental feed that is experienced in NZ, may increase the profitability and reduce the risk of complementary forages crops in moderately intensive NZ dairy systems.

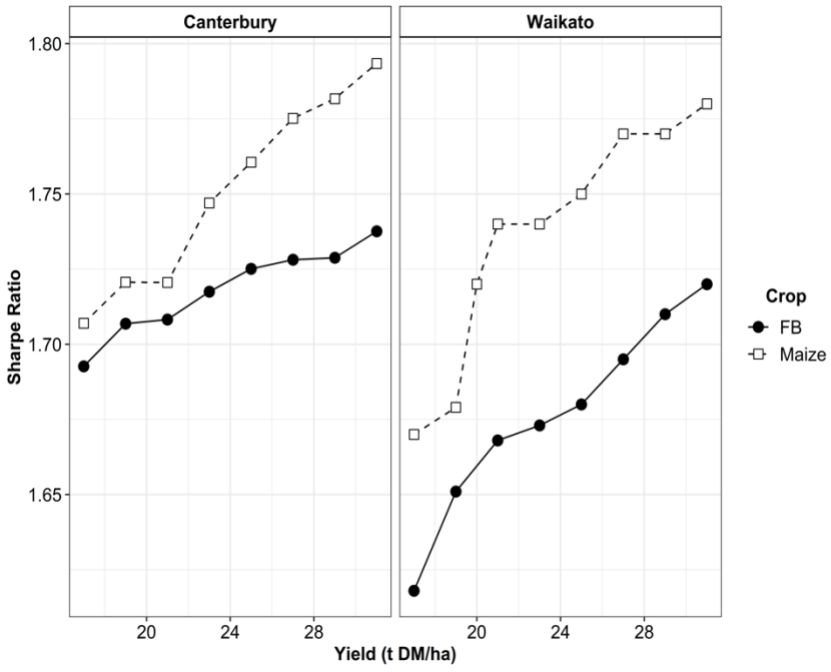


Figure 8.5. The effect of dry matter yield (DM) of either fodder beet (FB) or maize silage (Maize) crop grown on and irrigated (Canterbury) or an unirrigated (the Waikato) system, on the Sharpe ratio between mean return on asset (ROA) and the standard deviation of ROA.

Differences between regions reflect system intensity. The irrigated Canterbury scenarios imported 5% more feed and supported greater stocking rates which increased milk production per ha, and ROA by 7%, compared with dryland Waikato scenarios. However, the variation of ROA between all scenarios was minimal, ranging from 10 to 12%. The low correlation between ROA and system intensity outlined by Shadbolt (2012) explains the limited ROA variation between scenarios in the current modelling exercise. Variation of commodities (feed milk, fuel, etc.) and climate may cause greater ROA variation than changes to system management (Shadbolt et al., 2017). However, the minor (2%) variation of EFS, ROA and Sharpe ratio predicted between scenarios may still be economically meaningful, long-term. The range of ROA across seasons and market fluctuations may prove more valuable for describing the competitive advantage (longevity) of NZ dairy systems. While the mean ROA of a system 5 (>30% feed imported) farm in NZ between 2005 and 2015 was greater than a system 4 or a system 3 farm (7.4, 6.6 and 6.6 % respectively), the range of the ROA from the system 5 intensity farm was greater (-7.8 to 18.7%) than the range of ROA from a system 3 intensity farm (- 4.9 to 14.8%) (Wales and Kolver, 2017). In the current simulations, the standard deviation of ROA predicted for Canterbury scenarios increased 6% compared with the Waikato scenarios. Model predictions for ROA were 2-3% greater than those reported by others and the average ROA for owner-operator systems in 2016-17 (9.6%) (Shadbolt et al., 2017; Wales and Kolver, 2017; DairyNZ, 2018b). Differences between predicted and actual ROA values are probably explained by the variation of the input and output values used. While regional differences between the Base scenarios suggest that minor intensification of dairy systems may be economically justified, the interaction between local weather and local input costs (and their fluctuation between season), in addition to environmental outcomes, should also be considered.

### 8.5.2 Ruminal acidosis from FB

Simulation of acidosis in FBAC was designed to reduce DMI by ~5% and result in a 1% death rate when FB was introduced to the diet in early and late lactation. A lower feed intake (to simulate the effect of low rumen pH) was imposed by increasing the substitution rate from 1.0 to 1.2 kg DM herbage/kg DM of FB. The feasibility of using high substitution rate assumptions is supported by earlier observations of SARA in lactating dairy cows (Khafipour et al., 2009a; Khafipour et al., 2009b) but are only an estimate of the potential effect on animal production and farm profit. While the acidosis level was kept consistent across the two

regions, the Canterbury scenario was predicted to experience a greater reduction of milk yield and profit compared with the Waikato scenarios (Table 8.2). The variation of stock numbers and the subsequent effect on feed expenses probably explain the variable risk response to FBAC across the two regions. Fatalities from FBAC reduced the need for imported silage which was more expensive in Canterbury compared with the Waikato. However, the lower livestock sales in FBAC was due to the limited ability to cull poorly performing animals. The increased fatality rate due to feeding FB in the FBAC scenario will reduce the genetic progression of the herd, although this effect has not been considered in NZ pastoral dairy systems. The continued use of FB over subsequent years may also increase the incidence of acute and SARA. The exposure of the rumen epithelium to low pH (< 5.6) can cause long-term structural damages which reduces the absorptive capacity and increases the susceptibility of dairy cows to SARA (Allen, 1997; Dohme et al., 2008; DeVries et al., 2009). The decline of liveweight due to SARA was a secondary factor contributing to the low EFS predicted for the FBAC scenario, as the cost to restore 1 kg of liveweight was $ 0.95 (DairyNZ 2016-17 Economic Survey). Further evaluation of the long-term costs of reduced genetic selection and impaired animal welfare can occur when FB is fed to dairy cows is needed.

The FBAC scenario is not an accurate description of RA and SARA's monetary cost, which can occur when FB is used to support lactation. The primary limitation of the current model is the paucity of data describing the rate, duration and severity of both RA and SARA across the herd. Quantitative responses to acidosis vary with the severity of insult and are often confined to mismanagement of FB, such as; allocation, identifying poor performing cows, or poor transitioning methods. However, other biological factors such as; climate, management, individual susceptibility and variation of crop yield across the paddock, will also increase the risk of SARA (Owens et al., 1998; García et al., 2007; Gibbs, 2014). Plaizier et al. (2008) estimated that the cost of ruminal acidosis was $400 (USD) per cow each season, due to lost milk production (-2.7 kg milk/cow per day). That estimate may have risen, had consideration been given to the increased costs of poor animal health, costs associated with greater culling, or reduced capital value due to stock fatality. In comparison, the cost of acidosis in the current study was conservative, and acidosis only accounted for $108 (NZD) per cow in Canterbury and $50 per cow in the Waikato. However, the use of empirical data to quantify the tangible effects of SARA from a PRG x WC herbage and FB diet in NZ dairy systems will improve the accuracy of model predictions.

### 8.5.3 Comparison of forage crops

The opportunity cost of complementary forages may have a greater effect on EFS and ROA than DM yield. Despite the greater DM yield of FB, EFS was greater for the MSC scenarios at both locations. The growing season of FB was approximately 50 days longer than for maize silage (Chakwizira et al., 2014) and because conditions are too wet and cold in Canterbury to sow a new pasture before winter, the FB area is left fallow until spring (Edwards et al., 2014a). However, it is worth pointing out that the late winter to early spring period is the most desirable time for the ground to be fallow, as solar radiation and potential plant growth are low (Fletcher et al., 2011). By comparison, the maize crop was harvested for silage in early autumn, with the paddocks returned to pasture by April, and available for the first grazing rotation in spring (Clark et al., 2001; Densley et al., 2001). Following autumn grazing, the FB area could be re-sown in cereal silage (such as oats: *Avena sativa* L.), to minimise the time the paddock is fallow (Malcolm et al., 2016). While the inclusion of a second supplement to the FBC scenario would have caused ambiguity of response variables in the current modelling, the rotation of autumn grazed FB into a cereal silage catch-crop may require further investigation.

Fodder beet increased the crop duration (between the last grazing of the original sward and the first grazing of the newly established sward) by 112 days compared with that of maize silage. While the mean DM yield of FB exceeded that of maize silage, the longer crop duration reduced pasture growth in the following season, a factor which is often omitted in profit analysis of complementary forages (Fletcher et al., 2011). The advantage of grazing new pasture in spring may also explain the numerically greater pasture cover, and peak milk production predicted from the MSC scenario in the Waikato (Figure 8.2, Figure 8.4). Model predictions suggest the shorter crop duration of maize silage compared with that of FB was of greater advantage to ROA and EFS than DM yield/ha of the crop (Figure 8.6).

Differences in crop area between FBC and MSC may also explain why EFS declined with the FB scenario. Fodder beet represented only 7% of the milking platform in FBC scenarios while 8% of the milking platform was grown in maize silage in the MSC scenarios. The average crop yield drove the area of maize silage and FB at each location. The scenarios were designed based on industry recommendations that intake of FB was < 40% of DMI during late lactation (Dalley et al., 2019; Waghorn et al., 2019) and < 30% DMI in early lactation (Chapters 3, 4, 5, & 7). The allocation of maize silage did not exceed the 6.5 kg DM/day recommended for maintaining the protein requirements of lactating cows (Kolver et al., 2001). While the starch

content of maize silage can also cause ruminal acidosis, the development of SARA from FB presents a far greater risk, due to the large proportion of WSC and intake of FB must be restricted to maintain animal health.

Despite minimal differences in milk production per cow, MSC produced more MS than all other scenarios in the Waikato. The scenarios were designed to meet energy demands of stock as required, and feeding policies were very similar between scenarios; thus, the milk response to supplements are also similar (Bargo et al., 2003). However, DMI and milk production were both greater in the MSC scenario compared with all other scenarios in September. In all scenarios, PRG x WC was grazed 'to demand' which was calculated each day based on the current sward mass available and the daily growth. Average PRG x WC cover of the MSC scenario in the Waikato region was much greater than all other scenarios in August to September, reflecting the shorter growing season of the maize crop and the greater accumulation of spring PRG x WC herbage compared with FBC. The increased cover of PRG x WC herbage in the Waikato MSC scenario may also have enabled cows to graze more selectively, as post-grazing residual cover increased compared with Base or FBC (Table 8.3). However, the model did not predict that the ME content of the diet (averaged per season or month) would decline with greater post-grazing cover. The greater milk production achieved from MSC compared with FBC suggests that a shorter duration of crop sequence may be of greater importance for improving profit margin, than the DM yield of crop or the milk response to supplement.

### 8.5.4 Utilisation

The system's resilience is defined by not only financial efficiency as discussed above but also technical attributes such as maximising utilisation of feed. On average, the MSC diet contained a greater percentage of grazed PRG x WC herbage compared with FBC or FBAC (Table 8.3). Dietary inclusion of PRG x WC herbage was also slightly greater in Canterbury than in the Waikato (73.3 versus 74.5% of DMI). The processes which define the utilisation of supplemental feeds, such as conservation, harvesting or feeding out to stock are a key concept for production efficiency (Shadbolt et al., 2017). The increase of herbage utilisation may be more important to profit margin than the immediate milk response to supplement (Dillon et al., 2002). Furthermore, Ramsbottom et al. (2015) reported that EFS declined € 450/ha when the amount of herbage grazed was reduced from 8.4 to 7.0 t DM/ha. In the current simulations, the amount of grazed PRG x WC herbage ranged from 12.2 to 13.8 t DM/ha while

pasture yield ranged between 13.0 to 15.4 t DM/ha (Table 8.3). Therefore, the capacity to improve herbage utilisation by grazing is limited in the current scenarios, without increasing the amount of imported feed.

The utilisation rate and the DM yield of the crop are also important contributors to the system's economic and technical efficiency. While grazed FB was highly utilisable (90%) (Saldias and Gibbs, 2016), utilisation of harvested FB was assumed to be the same as maize silage (75%), due to a paucity of information describing the efficiency at which FB is harvested, stored and fed-out to stock. The FBC scenarios were designed for FB to be grazed up to 5 kg DM per cow in autumn and residual crop was harvested in spring at a lower rate of utilisation, which accounted for the loss of any residual leaf and crop residue left in the paddock following harvest. In comparison, excess maize silage can be stored and fed to cows in the following season, or sold based on current market values. Variable FB yields reduced the Sharpe ratio compared with MSC, reflecting a combination of restricted feeding management preventing the storage of FB longer-term, and the increase of crop cost when FB is harvested and fed-out during spring compared with *in situ* grazing of FB.

## 8.6 Conclusion

Model predictions suggest a greater advantage to growing maize silage on the milking platform than importing maize silage or growing FB to support early and late lactation milk production. Across both regions, MSC better complemented NZ’s pasture-based systems by improving milk production, profit and reducing business risk, due to a combination of shorter crop sequence and greater feeding flexibility compared with FBC. While there are some limitations of the model for evaluating RA and SARA from FB, a relatively minor occurrence caused a substantial decline of income that increased with system intensity. Given that EFS, ROA and risk were all improved by growing maize silage on the platform, there appears to be little advantage for growing and feeding FB to support lactation.

# Chapter 9
# General Discussion

# A rumen, animal and farm systems evaluation of fodder beet when used to supplement ryegrass during lactation

## 9.1 Introduction

Large DM yields and high crop utilisation have led to the wide-spread integration of fodder beet (FB) crops into New Zealand dairy systems to complement seasonal herbage growth. However, the integration of FB at the system level is associated with increased tactical, biological and financial risk (Chapter 2). The high water-soluble carbohydrate (WSC: 73%) and low fibre (< 20% DM) content of FB bulb (DairyNZ, 2017) are factors which promote ruminal acidosis and may impair animal welfare (Owens et al., 1998; Plaizier et al., 2008). Hypothesis #1 (Table 1.1) tested whether the large WSC content of FB bulb could complement the nutritional composition of ryegrass, increase early-lactation milk production and the poly-unsaturated fatty acid (PUFA) content of milk. The lack of milk response in cows supplemented with FB in Chapter 3 & 4 suggest hypothesis #1 should be rejected.

Sub-acute ruminal acidosis (SARA) is characterised by the rapid accumulation of volatile fatty acid (VFA) from WSC fermentation, which reduces ruminal pH, microbial activity, buffering capacity, rumen motility and milk production. Therefore, the hypothesis #2 tested whether supplementation of ryegrass with FB would reduce ruminal pH and increase fermentation kinetics, we also hypothesised that FB would alter fermentation pathways leading to increased synthesis of butyrate and propionate. The results of Chapters 4, 5 & 6 support hypothesis #2 regarding ruminal pH, fermentation rate and the increased synthesis of butyrate. However, the response propionate and total VFA concentrations when FB was included in the diet were inconsistent between Chapters 4, 5, 6, & 7, and the consequences or reasons for these inconsistencies require further evaluation.

Structural damage to the rumen epithelium can occur which reduce the absorptive capacity of rumen long-term and increase the ruminant's susceptibility to SARA when ruminal

pH is < 5.6 (Dohme et al., 2008; DeVries et al., 2009). The severity of SARA varies between individuals and is defined by the duration that rumen pH is below 5.6 (severe SARA) or 5.8 (mild SARA) (Garrett et al., 1999; Brown et al., 2000; Gozho et al., 2005). However, low ruminal pH conditions can also cause lactic acid-producing lactobacilli to increase, leading to a downward spiral of ruminal pH (pH < 5.0), which the animal is unable to correct. Translocation of rumen endotoxin across the rumen epithelium into the portal circulation may also cause liver mediated inflammation (laminitis, mastitis, and ruminitis) (Nocek, 1997; Owens et al., 1998; Plaizier et al., 2008). The short- and long-term implications of SARA on animal production and welfare are considerable, and the research presented in this thesis suggest that SARA caused by supplementing ryegrass with FB may have profound negative implications to animal health and welfare.

Feeding management of FB is an essential tool for reducing the risk of ruminal acidosis and improving animal welfare of cows fed FB. Cows must be transitioned to FB gradually and may require greater than 20 days to acclimatise to a perennial ryegrass and FB diet (Chapter 4 & 5). In New Zealand grazing systems, FB is either grazed *in situ* (usually during late-lactation - winter), or bulb is defoliated, harvested and stored above ground to supplement herbage in spring (Gibbs, 2014). Controlling the allocation of FB to individuals within the herd is needed to prevent ruminal acidosis. However, FB feeding practices are reliant on each animal consuming the same amount of FB each day. Hypothesis #3 - that industry-approved methods of transitioning to FB would reduce the risk of SARA during lactation was tested in Chapter 4. However, individualised feeding practices suggest the intake of FB and herbage varies between individuals and between days which caused two out of eight animals to experience severe SARA, which suggests that hypothesis #3 should be rejected. It may not be realistic to expect group feeding strategies to maintain consistent FB intakes, and, likely, the true impact of SARA from FB in pastoral dairy systems of NZ is greater than currently realised. Furthermore, certain individual animals respond differently to FB consumption and may be more susceptible to SARA than others. The factors associated with individual responses to FB have not been formerly considered in New Zealand and are required to further develop feeding strategies which improve animal welfare when FB is included in the diet.

Chapter 5 was a continuation from Chapter 4, using the same animals following 20-days of transitioning to a FB and grazed ryegrass diet. Chapter 5 tested #4 - that the decline of ruminal pH caused by feeding dairy cows FB would reduce ruminal degradation of ryegrass,

grazing time and aspects associated with oral processing. The results of Chapter 5 supported hypothesis #4, which may explain why the milk response, when FB supplements ryegrass, is limited.

Chapter 7 tested hypothesis #5: that a combination of amount, time, and frequency could be used to formulate a diet containing FB and ryegrass that would optimise milk production, limit the risk of SARA and reduce animal discomfort. Pareto front analysis of model predictions indicates that minimising FB allocation (< 25% DMI), feeding dairy cows FB twice-daily, and increasing the herbage allocated (fed once-daily), can improve the milk response to FB and provide the best trade-off between improving milk production and reducing animal discomfort from FB.

The final hypothesis tested how growing FB on the milking platform to support lactation affected financial, production, environmental and risk outcomes of the whole-farm-system compared with growing or importing maize silage (Chapter 8). While Fodder beet was competitive compared with imported maize silage under irrigated conditions, profit was similar, and the financial risk of the FB scenarios increased compared with growing maize silage on the platform. FB reduced profit and increased risk under dryland conditions compared with importing maize silage while growing maize silage on the platform improved profit outcomes and reduced risk. Based on these results, we accepted the final hypothesis, but we could not address the impact on enteric methane ($CH_4$), nitrate leaching ($NO_3$) and soil conditions.

This discussion summarises the results of a multifaceted thesis which has explored the dynamics of rumen fermentation, intake behaviour, production, farm profit, environmental outcomes and business risk when grazing dairy cows are supplemented with FB. The purpose of this general discussion is to discuss the primary factors which may prevent the successful integration of FB at the rumen, individual animal and farm system level and to summarise the findings of this research concerning the initial hypotheses and objectives outlined in Chapter 1 (Table 1.1).

## 9.2 The rumen

### 9.2.1 Epithelial structure

The stratified squamous epithelium of the rumen is comprised of four functionally divergent strata which enable transportation, absorption, metabolism and provide protection (Figure 8.1), these include the: stratum corneum, stratum granulosum, stratum spinosum and stratum basale (Graham and Simmons, 2005). The stratum corneum is directly adjacent to the lumen and contains highly cornified keratinocytes that act as a protective barrier (Steele et al., 2009). The stratum spinosum and stratum basale have large numbers of mitochondria responsible for epithelial metabolism and ketogenesis (Baldwin, 1998). Within the stratum granulosum, tight gap junctions (desmosomes) exist between cells, providing a barrier for diffusion between the rumen and the portal circulation (Graham and Simmons, 2005). Low ruminal pH causes functional changes to the epithelium by reducing the integrity of these tight junctions and increasing permeability of the rumen wall (Plaizier et al., 2008). Penner et al. (2010a) reported one marginal SARA challenge (< 5.8 for 111 min/day and nadir pH 5.48) did not affect barrier function in sheep when induced three hours before death. In Chapter 4, grazing dairy cows experienced a similar SARA challenge over several days, which was exacerbated by feeding moderate FB proportions (38% of DMI). The severe and continuous SARA experienced in 1/4 cows fed FB bulb (pH < 5.5 for >180 minutes/day) may have reduced the integrity and absorptive capacity of the rumen epithelium (Bull et al., 1965; Aschenbach et al., 2011).

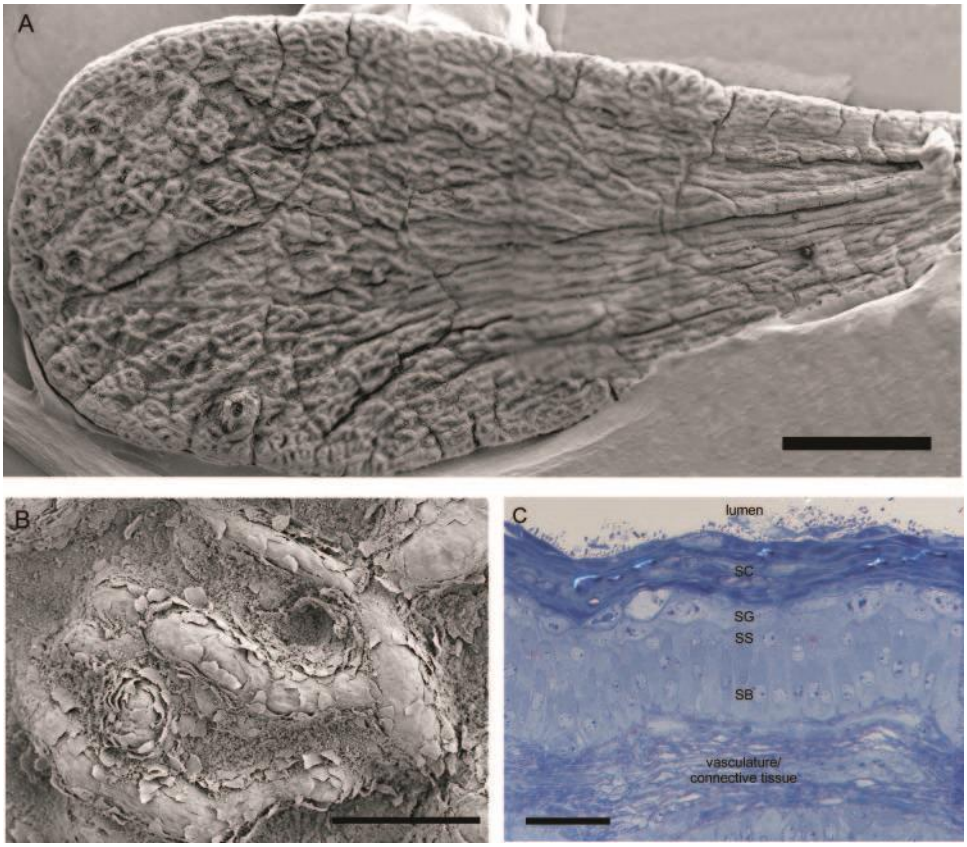


Figure 9.1. An electron micrograph of A: a complete dissected papilla. Scale bar = 2mm. B: magnification of the epithelial surface, displaying cells at the ridges in the process of sloughing from peripheral stratum corneum note: the presence of microflora obscure the epithelial surface between ridges. Scale bar = 100 μm. C: transmitted light micrograph of a toluidine-blue-stained section of a papilla showing the position of the stratified epithelium: Stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS) and stratum basale (SB). Scale bar = 20 μm *(Graham and Simmons, 2005)*.

To maintain ruminal pH, functional changes of epithelial metabolism can occur rapidly and in response to local (at the apical membrane) concentrations of specific VFA. The absorption of VFA is estimated to neutralise 53% of protons in the rumen (Allen, 1997) and is the primary mechanism for maintaining ruminal pH. Morphological and metabolic adaptions can increase the absorptive capacity of the epithelium and is a natural postpartum adaptation to increase epithelial surface area for absorption (Storm et al., 2012). Morphological changes, such as; increased blood flow, the proliferation of the epithelium and extension of papilla length, occur over a variable period (1 day for blood flow and between 16 days and 8 weeks papillae proliferation) (Dirksen et al., 1985; Cassida and Stokes, 1996; Malhi et al., 2013; Dieho et al., 2016). However, the expression of genes associated with VFA transport can increase 219% within the first week following a diet change (Etschmann et al., 2009; Dieho et al., 2016). Despite the rapid ability of the rumen wall to adapt to increased concentrations of VFA, dairy

cows still experienced low ruminal pH following 20 days of adaptation to FB (Chapter 4). The short adaptation to FB in Chapter 4 may not have been sufficient for structural adaptations of the rumen epithelium to occur. The development of SARA in lactating dairy cows and some instances, outside of the initial transitioning to target FB allocation (Chapter 4) and by others (Waghorn et al., 2018; Waghorn et al., 2019), highlight the need for more extensive analysis of rumen epithelial structure and development associated with dietary adaptation to a mixed FB and ryegrass diet.

The absorption of VFA from the rumen occur via three processes; passive diffusion, $HCO_3^-$ dependant and $HCO_3^-$-independent exchange (Gäbel et al., 2002; Dijkstra et al., 2012). Bicarbonate-dependant and bicarbonate-independent mechanisms remove the majority of acetate and some propionate form the rumen. In comparison, butyrate and only a minor proportion of acetate are passively diffused from the rumen (Aschenbach et al., 2009). The extent of VFA removal from the rumen via passive diffusion and bicarbonate dependant exchange increases relative to luminal acidity and apical VFA concentrations (Aschenbach et al., 2009). Fractional absorption rates (per h) of acetate, propionate and butyrate increased 40, 48 and 67% when pH declined from 7.2 to 4.5 *in vivo* (Dijkstra et al., 1993). In Chapter 4 & 5, cows supplemented with FB experienced lower ruminal pH but experienced lower total VFA concentrations than cows fed the HO diet. The low ruminal pH of cows supplemented with FB may have led to the underestimation of total VFA concentrations due to increased VFA flux not detected by the 4 h rumen sampling intervals. Yet, the presumed increase of VFA flux does not account for the decline of ruminal pH at midday, which also coincided with increased concentrations of lactic acid. However, diet and sampling time of lactate was not significant ($P > 0.1$) by the third stage of adaptation. While the specific mechanisms responsible for the decline of ruminal pH in cows fed FB are not immediately clear, the negative impact to rumen function (Chapter 4, 5 and 7) likely explain the low milk response to FB. Further research of the dynamics associated with VFA flux in cows fed FB may help to clarify the response of grazing dairy cows supplemented with FB.

### 9.2.2 Fermentation pathways

The addition of FB to a perennial ryegrass-based diet consistently increased ruminal concentrations and pools of butyrate (Chapter 4, 5 & 6). Butyrate concentrations increased 19% when FB was 38% of DMI (Chapter 4; *in vivo*) and 12% when FB was 35% of the diet (Chapter 6; *in vitro*). Butyrate is lipophilic and is metabolised extensively (80-90%) by the

rumen epithelium, creating a concentration gradient that promotes the rapid diffusion of butyrate across the phospholipid bilayer of the rumen wall (Dijkstra et al., 1993; Penner et al., 2009b). Consequently, the removal of butyrate from the rumen occurs at a faster rate than acetate or propionate (Dijkstra et al., 1993; Penner et al., 2009b). However, the majority of ruminal VFA exist in a dissociated state ($VFA^+$). Between 1-6% of VFA (pH 6.0-6.8) are undissociated (HVFA) and can passively diffuse across the rumen wall (Owens et al., 1998; Dijkstra et al., 2012). Once diffused, cytosolic HVFA is dissociated and either metabolised by epithelial cells or transported into the portal circulation (Kristensen et al., 2000). The remaining $H^+$ must then be transported out of the cell by Na+/H+ exchangers (NHE), to prevent intracellular acidity (Aschenbach et al., 2011). The NHE is either located on the apical or basolateral regions of the cell, and it is not known whether the passive diffusion of protons from the rumen is permanent. However, apical transport of $Na^+/H^+$ has been reported and may reduce apical alkalisation caused by $HCO_3^-$ dependant transport (Laarman et al., 2013; Penner, 2014). Previous studies have suggested other sucrose-rich feeds such as molasses increase butyrate formation which may improve ruminal pH and reduce the risk of SARA compared with starch-based feeds (Plöger et al., 2012; Schlau et al., 2012). The research presented in this thesis indicates that while FB increases butyrate in the rumen, ruminal pH is still reduced compared with a herbage only diet.

While the molar proportion of butyrate in the rumen is low, it is the most dynamic VFA, varying from 5 to 30% of total VFA and is associated with several signalling pathways involved in proliferation, apoptosis and differentiation in the human colon. Butyrate can reduce the progression of colon cancer and improve barrier function (Scheppach et al., 1995; Plöger et al., 2012) and has anti-inflammatory properties that are beneficial to human health (Schröder et al., 1999). However, the synthesis of butyrate in ruminants is substantially greater than the human colon, and the potential advantage to ruminant metabolism is less clear (Gäbel et al., 2002). Butyrate increases the absorptive capacity of the rumen by stimulating the differentiation and proliferation of epithelial cells (Sakata and Tamate, 1978), portal blood flow (Storm et al., 2011), papillae growth (Malhi et al., 2013) and molecular upregulation (Dijkstra et al., 1993; Kristensen et al., 2000; Penner et al., 2010b). When intra-ruminal infusion of butyrate increased from 4.0 to 37.4 mmol/L, epithelial circulation increased 47%, and the disappearance of propionate increased by 11% (Storm et al., 2012). However, butyrate can also cause excessive differentiation and sloughing of epithelial cells (see Figure

9.1) or para-hyperkeratosis, which can reduce the absorptive capacity of VFA from the rumen. Para-hyperkeratosis can cause a welfare challenge which is long-term by reducing ruminal pH, the absorption of VFA for ruminant metabolism, and enhancing the risk of SARA re-occurrence (Dohme et al., 2008; DeVries et al., 2009). While it is unlikely that butyrate concentrations of cows fed FB (< 40% DMI) will cause substantial damage to the epithelial structure, research of cows fed greater allocations of FB, and the ¼ cows which were susceptible to SARA when fed FB, is warranted.

The results of this thesis suggest a gradual and more prolonged transitioning and adaptation to FB are vital for the successful integration of FB at the rumen-scale. While traditionally SARA is characterised by the accumulation of VFA, the observed increase of lactic acid *in vitro* and *in vivo* suggest a combination of VFA and lactic acid reduce rumen pH of cows fed FB. While altered fermentation pathways increase the microbial synthesis of butyrate, the net effect of elevated butyrate on epithelial metabolism, structure and hepatic metabolism does not appear to be sufficient to improve the ruminal pH of grazing dairy cows supplemented with FB.

## 9.3 The individual animal

### 9.3.1 Epithelial function

Specific functional adaptation of the rumen epithelium to a FB diet may also account for some of the individual variations observed in Chapter 4 and previous studies (Waghorn et al., 2018; Waghorn et al., 2019). Functional differences of the epithelium are associated with individualised risk of SARA in cows fed a total mixed ration (Bevans et al., 2005; Penner et al., 2007; Gao and Oba, 2014; Gao and Oba, 2016). The response of individual cows to a readily fermentable diet is multifaceted, reflecting several genotypic, phenotypic, metabolic and microbial factors (Cassida and Stokes, 1996; Penner et al., 2007). Observation of the mechanisms involved suggests that cows which are less susceptible to SARA experience lower VFA concentrations (and rate of accumulation) in addition to the increased rate of VFA absorption (Gao and Oba, 2016). The expression of NHE was upregulated 176% in steers which displayed SARA resistance when fed a high-grain diet compared with SARA-susceptible steers which experienced lower rumen pH in response to a SARA challenge (Schlau et al., 2012). In sheep that were not responsive to a SARA challenge, the apical absorption of butyrate and acetate increased compared with sheep, which experienced an acute response to a SARA

challenge (Penner et al., 2009a). However, the VFA absorption rate was not greater in dairy cows identified as high risk of developing SARA (Gao and Oba, 2016). Inconsistent results may relate to the varying divergence between animals defined as either high or low risk for developing SARA. Furthermore, research of the mechanisms underpinning the individual animal response to feeding or grazing lactating or non-lactating dairy cows on FB is needed.

### 9.3.2 Milk response to FB

In agreement with previous indoor studies, the milk response to FB from grazing dairy cows found in this research is limited. In the first experiment presented in Chapter 3, the substitution of poor-quality ryegrass with harvested FB bulb (FBB) did not improve milk production despite the greater apparent ME intake of the diet, compared with un-supplemented cows. The reproductive state of ryegrass grazed by the FBB treatment prevented the full rejection of hypothesis #1; that FB is advantageous to milk production. Since the publication of Chapter 3, further and a more recent study of milk production of cows fed FB, also indicate that feeding moderate amounts of FB does not improve milk production compared with a herbage only diet (Waghorn et al., 2019; Pacheco et al., 2020). Model predictions (Chapter 7) and empirical research reported in this thesis (Chapter 4) and elsewhere (Dalley et al., 2019); indicate the milk response to FB supplement is reduced by sub-optimal rumen pH when lactating dairy cows are fed > 38% of their daily intake as FB. Low ruminal pH inhibits the microbial degradation of structural carbohydrates such as cellulose and hemicellulose (De Veth and Kolver, 1999; De Veth and Kolver, 2001; Krajcarski-Hunt et al., 2002). The decline of microbial degradation due to low ruminal pH reduced the *in sacco* DM disappearance of ryegrass by 19% in cows fed a mixed herbage and FB diet compared with those fed HO (Chapter 5). In agreement with Dalley et al. (2019), model predictions from Chapter 7 suggest that FB may provide a small advantage to milk production during early lactation. Alabi (2019) concluded that low ruminal pH caused high substitution rate (1.5 kg DM herbage/kg DM FB) and low milk response (-0.63 kg milk/kg DM of FB) to a herbage-based diet supplemented with 20% FB during early lactation. The milk response and substitution rate of Alabi (2019) contrast to those observed in Chapter 4, in which the milk response was low and variable (0.39 $\pm$ 0.29 kg/kg DM FB), but substitution rate was also low and variable (0.31 $\pm$ 0.22). However, evaluation of DMI from energy output in milk, liveweight gain and maintenance suggests the substitution rate of ryegrass from FB may have been greater than previously anticipated at 1.08 kg DM ryegrass/kg DM of FB. While cows fed FB and herbage in

Chapter 4 experienced marginal SARA, the results of Alabi (2019) may suggest a lower allocation of FB is needed to reduce risks of SARA further, and which appeared to limit animal production to a greater extent than that observed in Chapter 4.

The variation between Chapter 4 and Alabi (2019) may also reflect group feeding of FB which creates competition (increases intake rate) amongst the herd (Alabi, 2019), compared with feeding FB to individual cows (Chapter 4). Proudfoot et al. (2009) have similarly observed that group feeding conditions enhance intake rate and risk of SARA from grain-based supplements. The ability to feed FB to individual cows rather than a herd may be more important for preventing SARA than increasing herbage allocation. Furthermore, Chapter 7 indicate that increasing the frequency of FB feeding may reduce the VFA load in the rumen pH. Macmillan et al. (2017) have also reported that increased frequency of supplementation (of grain-based feeds) increases cows' ruminal pH when exposed to a SARA challenge. While the use of herbage allocation as a management technique for preventing SARA from FB is not consistent, increasing supplementation frequency may help to reduce the individual response to FB supplementation.

The use of feeding behaviour to identify cows experiencing a SARA challenge has been explored by inducing SARA with cereal grains. While reduced time spent ruminating and grazing are consistent with the impaired rumen function commonly experienced during severe SARA (pH <5.5), (DeVries et al., 2009) reported that rumination time increased the day following a SARA challenge. In Chapter 5, we hypothesised the increased rumination, and chewing intensity experienced by cows fed FB may have been a compensatory mechanism to improve digesta particle comminution of cows which experience a mild SARA challenge. The finding that FB increased rumination and chewing intensity while ruminating is unique as rumination, and chewing activity is generally expected to decline in cases of severe SARA (Owens et al., 1998). However, we are not the first to note that rumination may increase in response to low rumen pH (Williams et al., 2006). The observed increase of rumination and chewing intensity during periods of low rumen pH may not only aid particle breakdown but may also promote the secretion of saliva containing $PO_4^-$ and $HCO_3^-$ buffers (Cassida and Stokes, 1996; Maekawa et al., 2002). Further evaluation of the grazing dynamics and salivation of individual cows supplemented with FB may be required.

### 9.3.3 Milk composition.

During early lactation, feeding FB changed milk fatty acid (FA) profiles, which indicate altered biohydrogenation of the rumen. During rumen fermentation of plant lipids, PUFA is bio-hydrogenated (BH) to reduce their toxicity to rumen microbes (Bauman and Griinari, 2001). The majority of unsaturated FA in milk is of dietary origin which has escaped ruminal hydrogenation and enters the mammary gland via the circulation. Some UFA such as conjugated linolenic acid (CLA *cis* 9 *trans* 11) is synthesised endogenously from vaccenic acid (C18:1 *trans* 11) by delta-9-desaturase in the mammary gland (Chilliard et al., 2000). In Chapter 3, feeding FB reduced the milk content of CLA *cis* 9 *trans* 11 synthesised endogenously, presumably due to the reduction of poly-unsaturated substrate available in FB bulb. Despite reduced PUFA content of the diet, FB did not reduce the PUFA content of milk significantly in the second experiment (Chapter 4), which may provide some support for the initial hypothesis, and the effect of low ruminal pH on rumen fermentation. Saturated fatty acids are synthesised *de novo* within the mammary gland using primarily acetic (85%) and some butyric (15%) units, by Acetyl CoA Carboxylase and Fatty Acid Synthase (Chilliard et al., 2000) In Chapter 3, the content of SFA in milk increased 9.18% when FB supplemented ryegrass. However, in Chapter 4, the SFA content of milk increased by only 3.1% ($P < 0.05$) in response to FB supplementation in Chapter 4. However, the average SFA content of milk in Chapter 3 was similar to that observed in Chapter 4 (81 vs 73 g FA/100 g FA). While changes to the fat composition of milk were modest in Chapter 4, the increase of medium-chain and saturated fats (lauric; C12:0, myristic; C14:0 and palmitic acid; C16:0) still support the conclusion that FB increases *de novo* synthesis of FA.

Supplementing grazing dairy cows with FB at either 30 or 40% of daily DMI does not appear to provide any advantage to milk production. However, changes to biohydrogenation products formed in milk suggest altered rumen microbial activity, and further research of microbial communities within the rumen of cows supplemented with FB is still needed. While simulation modelling predicted milk production from FB could be improved by feeding small allocations (<25%) during early lactation, the empirical research from this thesis and others (Alabi, 2019; Dalley et al., 2019), indicates the milk response to FB is still limited when included at 20% of daily intake. Research is needed to establish the upper limit of FB intake further when FB is fed to the herd. However, due to the individual response and decline of *in sacco*

DM disappearance reported in Chapter 5, it is recommended that FB intake should not exceed 30% of DMI, to reduce the risk SARA in lactating dairy cows.

## 9.4 Farm system and herd level

### 9.4.1 Herd intake of fodder beet

Group/herd feeding strategies during FB transitioning are reliant on individuals within the group achieving consistent intake of FB between days. In reality, FB consumption varies considerably between animals which may increase the allocation of FB and the risk of SARA to the remaining herd (Chapter 4 and discussed in Chapter 7). The coefficient of variation (CV) of DMI intake averaged 24.1% in Chapter 4 and is reportedly greater for grazing dairy cows individually supplemented with a grain-based feed (CV of 32%) (García et al., 2007). Thus, the need to achieve a consistent intake of FB may not be realistic. The increased intake rate caused by competition may cause excessive consumption and ultimately can lead to aversive behaviours towards FB (Chapter 7), increasing the allocation of FB to the remaining herd. Aversive animals should be identified and removed. However, identification is not pre-emptive (Chapter 7) and as previously discussed, damage to the rumen epithelium can occur within days.

Comparison between Chapter 3 & 5 may provide some evidence that herd feeding may impair the animal response to FB compared with individual feeding. While the consumption of FB represented just 7% of daily activity, cows masticated FB approximately 864 chews/kg DM compared with 556 mastications/kg DM of ryegrass. This suggests that individually cows spend a considerable amount of time processing and breaking down FB bulb before ingestion, however, when fed FB as a group (or herd) the rate of intake appears to increase. In Chapter three, 30 cows consumed 4 kg DM of FB within 25 minutes (0.16 kg DM/min), when fed as a herd on a concrete feed pad, while each cow required ~2 hours to consume 5.79 kg DM of FB (0.05 kg DM/min) when fed individually in Chapter 5. In both cases, FB was chopped (by mixer wagon or by hand), and it is not clear how the increased rate of supplement intake will have affected the rate of digestion, VFA concentrations, and ruminal pH. While individualised feeding of FB in Chapter 5 may have improved the animal response to FB supplementation compared with feeding FB to the herd as reported by Alabi (2019) and by Dalley et al. (2019), rumen function was still impaired. Therefore, individual feed management of FB will not prevent sub-optimal rumen conditions.

### 9.4.2 Farm profit

Despite the high DM yields of FB crops (21-23 t DM/ha), whole-farm modelling (Chapter 8) suggests that growing FB on the milking platform is not advantageous to economic farm surplus (EFS: operating profit-adjustments) compared with maize silage. The low milk response to FB, extended duration of crop sequence (>12 months) and reduced annual DM yield of herbage following the FB crop, are primary limitations to EFS when FB is grown on the milking platform (Chapter 8). The use of FB bulb to support BCS gain during winter is challenging to replace in some areas of New Zealand that experience snowfall and heavy frosts. While swedes are an alternative option which retains their utilisation in colder climates over winter, they also present a significant animal health risk and research of alternative winter forage solutions which are practical and reduce animal welfare risk are needed.

The high DM yields achievable from FB also present a significant risk to the profit and production at the system level (Chapter 8). While Chapter 8 suggests the cost of growing FB on the platform and the opportunity cost from the extended crop duration does not outweigh the high cost of the establishment ($2,500/ha, $NZ) compared with maize silage, further cost comparisons of winter forages are needed. Initial reports suggested that growing FB for winter forage could reduce feed costs from 11-13 c / kg DM (for kale) to <10 c/kg DM (Gibbs, 2014). However, profit outcomes were based on achieving high yields and feeding FB *ad libitum* or to appetite of the diet. A recent study and industry recommendations are that FB should not exceed 60% of DMI (Waghorn et al., 2018), which mean additional silage is required to formulate a nutritionally balanced diet. Based on the average DM yield (23 t DM/ha; Chapter 8) and growing cost ($2,500/ha) of FB in Canterbury, the mean cost of FB is 10.9 c /kg DM, assuming 99% utilisation (Edwards et al., 2014a). The cost of silage on average is 28 $\pm$ 20 c/kg DM (mean $\pm$ sd); therefore, a 60:40 (FB: silage) diet will cost ~16.6 c/kg DM. In comparison, alternative winter forages such as kale cost ~$1,400 ha (Chapter 2) and should yield ~ 14 t DM/ha of which 89% is utilised (Edwards et al., 2014a), which will cost 11.2 c/kg DM and will increase to 14.6 c/kg DM if fed as an 80:20 (kale: pasture silage) diet. While this crude cost comparison does not include the increased area required for a kale crop compared with FB, formal cost analyses of various winter forages are needed, considering the increased risk of nutritional disorders associated with feeding cows FB.

### 9.4.3 Environmental pollution

The use of FB to reduce urinary N excretion and methane emissions are not supported by the research conducted during this thesis. Evaluation of the individual animal (Chapter 7) and system-scale (Chapter 8) implications of supplementing ryegrass with FB did not identify any advantage compared with maize silage supplement (Chapter 8) or a herbage only diet (Chapter 7 and 8). The results of Chapter 7 and 8 are consistent with the empirical study of lactating dairy cows supplemented with either FB or maize silage (Dalley et al., 2019). Similar to Chapter 7, Dalley et al. (2019) reported the amount of FB required to cause a meaningful reduction in urinary N concentrations, compared with cows supplemented with 25% maize silage, may exceed (> 40% DMI) what can 'safely' be fed to lactating dairy cows without causing SARA. Moderate supplementation of ryegrass with FB did not improve methane $CH_4$ emissions of livestock which is supported by *in vitro* (Chapter 6) and *in vivo* (Chapters 4 & 5) results. While previous research indicated FB could reduce enteric methane emissions (Jonker et al., 2016), the increased organic matter content of FB bulb increased total $CH_4$ yield *in vitro*. Enteric $CH_4$ emissions were not analysed in Chapter 4 or 5. However, the decline of propionate concentrations due to the reduction of rumen pH in cows supplemented with FB indicate that large amounts of FB are required to reduce $CH_4$ emissions and may be a consequence of SARA, a conclusion supported by mechanistic modelling (Chapter 7). Fodder beet is promoted to reduce urinary N concentrations and leaching in autumn and meet regional environmental regulations. However, the amount of FB needed to generate a meaningful reduction of urinary N concentration may impair animal health and sustainability of the system.

While DM yield of the crop is essential for reducing feed costs, *in situ* grazing of the high yielding crop may also deteriorate soil structure. Damage of the soil structure occurs through either compaction or pugging. While compaction is the gradual reduction of soil aeration within the sub-surface (0.05-0.1 m) of an unsaturated soil profile, stock treading on water-logged soils causes undulation at the soil surface (0-0.05 m) which reduce soil porosity and prevent drainage (Beukes et al., 2013). While compaction at the sub-surface is difficult to detect, the effects of pugging are noticeable and grazing winter forages at high stocking densities can cause long-term deterioration of soil conditions and reduce pasture production. Menneer et al. (2001) reported a single moderate (stock grazing for 1.5 hours) and severe (2.5 hours) pugging event on pasture (4.5 cows/100 $m^2$) reduced annual pasture yield by 21 and

45%, respectively. Drewry and Paton (2005) reported the compaction of soil below the cultivatable depth increased (0.10-0.15 m) with intensive wintering systems, reducing porosity, and hydraulic conductivity (the ease of fluid movement through pores) compared with lower stocking densities in Southland, New Zealand. Furthermore, the hydraulic conductivity measured in the hoof hollow areas declined 96% compared with that measured in hoof 'hump' areas (Drewry and Paton, 2005), which increases the surface flooding and horizontal erosion of sediment and nutrients from the soil into residing waterways (McDowell et al., 2003). Damage to topsoils, such as increased soil density and reduced aeration, can be corrected by cultivation. However, soil damage becomes more problematic and more difficult to correct when compaction occurs below the cultivatable depth (0.1-.015 m). Set stocking grazing management of all forage crops during winter are likely to deteriorate soil structure (Drewry and Paton, 2005). However, the high DM yields and restricted allocation required when feeding FB to stock *in situ* will increase stocking rate, which may exacerbate soil damage from winter grazing compared with lower-yielding alternatives such as kale. Other anecdotal reports of compaction due to the pressure in the sub-surface depth of the growing FB root may also alter the structural integrity of the soil. However, the potential damage to soil structure and the financial implications of subsequent yield reductions from replacement pasture following winter-grazed FB, are needed.

## 9.5 Conclusion

The rapid nature of metabolic adaptation of the rumen suggests the adaptation period used in Chapter 3 should have been sufficient to prevent ruminal acidosis from FB. However, pH was still limiting microbial function and reduced the degradation of herbage. Butyrate may play a role in improving the absorptive capacity of the rumen; however, the net effect on rumen structure, papilla growth and epithelial metabolism is not clear. While short-term changes of metabolism are an essential adaptive mechanism, the short 12-14 day adaption period used in the NZ dairy industry may not be sufficient for the structural adaptations of the rumen wall. Despite the gradual transition, specific individuals experience an elevated risk of SARA, which cannot be correctly managed at the herd level in null-infrastructure pastoral dairy systems common to New Zealand. While FB may be used to mitigate seasonal herbage growth, the high risk to animal welfare suggests alternative options for supplementing herbage should be considered if available.

To conclude; the use of FB as a supplement of pastoral systems in New Zealand, does not increase milk production, or milk quality, impairs rumen function and induces SARA which jeopardises the productive lives of cows. All of which increase the risk and do not improve profit outcomes of pastoral dairy production in New Zealand. While further research regarding soil conditions and epithelial adaptation may be required, the results indicate that supplementing ryegrass with FB is not advantageous at the rumen, individual animal or farm systems scale.

# Appendix A

## A.1 Chapter 3 as Published in The New Zealand Journal of Animal Science and Production (2018)



### Milk production and milk fatty acid composition of grazing dairy cows supplemented with fodder beet

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**Abstract**

A study was conducted to evaluate the effect of supplementing a perennial ryegrass-based diet with fodder beet on milk production and milk fatty acid (FA) composition, of dairy cows in early lactation. Sixty Friesian × Jersey cows, were blocked into six groups of 10 cows, and groups randomly allocated to three replicates fed either 18 kg DM/day of ryegrass herbage (H), or 14.4 kg DM/day of ryegrass herbage + 4 kg DM of harvested FB bulbs (FBB). Dry matter intake (DMI) was similar between H and FBB (15.0±0.77 and 14.2±0.48 kg DM/day respectively). Although milk yield tended to be greater for H than FBB (20.0 and 18.9 kg/day respectively; P=0.09); milk solids production was not affected by treatment (P=0.89). Supplementation with FBB increased the saturated (80.6 versus 73.2±0.39 g/100g FA; P<0.001) and medium chain milk FA (66.7 versus 56.2±0.783; P<0.001) content, compared with H. Under the conditions of the present study, our results suggest that, supplementing grazing dairy cows with FBB in early lactation, may not improve milk production and increases the saturated FA content of milk.

**Keywords:** fodder beet; milk; fatty acid; pasture

### Introduction

The large crop yields achieved by fodder beet, *Beta vulgaris* M., (FB) have led to its extensive use as a winter forage crop in New Zealand dairy systems. Compared with an alternative forage such as kale, FB can produce >20 t DM/ha (Chakwizira et al. 2013), which can be grazed or harvested and fed elsewhere or stored if necessary. This versatility is attractive for many farmers, as FB may be harvested to return the land to pasture and the FB fed to supplement the early lactation herbage supply. Although FB bulbs are high in metabolisable energy [ME: 11.8 MJ ME/kg DM (Clark et al. 1987)], studies undertaken internationally report minimal improvement of milk yield when FB is fed alongside various levels of protein (Fisher et al. 1994), or concentrates (Ferris et al. 2003). However, the milk response to supplementing a grazed herbage diet with FB has had little study.

Human food production from agriculture has traditionally focused on quantity. However, consumers are becoming increasingly aware of quality and associated health risks or rewards. For example, the concentration of polyunsaturated fatty acids (PUFA) such as conjugated linoleic acid (CLA: C18:2 cis-9, cis-12) or α-linolenic acid (C18:3 cis-9, cis-12, cis-15), have anti-carcinogenic properties and are associated with a range of benefits related to human health (Chilliard et al. 2000). Conversely, saturated FAs (SFA), have been linked with increased plasma concentrations of low density lipoprotein cholesterol, which may be a risk factor for cardiovascular disease (Shingfield et al. 2013). While herbage contains high concentrations of C18:3, and to a lesser extent CLA, ruminal bio-hydrogenation reduces their abundance in milk (Chilliard et al. 2000). Supplementation with starch has the potential to increase the PUFA content of milk as a result of reduced bio-hydrogenation at lower rumen pH (Kolver & De Veth 2002). While FB contains little starch, it is rich in soluble sugars, principally sucrose (Clark et al. 1987), which may also lower rumen pH and increase the PUFA content of milk. Consequently, the objective of this research was to determine the effect of substituting the herbage of grazing dairy cows with FB on milk production and milk FA composition.

### Methods

*Experimental site and design*

All animal treatments and measurements in this experiment were approved by the Lincoln University Animal Ethics Committee (#2016-30).

The experiment was conducted between the 10th and 25th of November 2016 at Lincoln University's Ashley Dene Research and Development Station in Canterbury (-43.65 º North, 172.33 º East), New Zealand. Sixty Friesian x Jersey (F9 J6) dairy cows were blocked into three replicate groups according to live weight (438± 3.1), age (3.6 ± 0.12 years) days in milk (DIM: 85 ± 4.8 days), and milk solids (MS: 2.02 ± 0.18 kg/day) and allocated to two treatments in a completely randomised design. Treatments were: perennial ryegrass (RG: *Lolium perenne* L.*)* and white clover (WC: *Trifolium repense*) sward, offered as an herbage only diet (H); or herbage + 4 kg DM/day of harvested FB bulb (FBB).

*Grazing management*

Fodder beet (cv. Rivage) was sown in October 2015, harvested commercially and stored five weeks prior to the study. Perennial ryegrass and white clover swards were grazed 4±1 weeks prior to the experiment and fertilised with 46 kg N/ha as urea. Prior to the experiment, all cows grazed a PRG WC sward supplemented with 3.5 kg DM/d

## A.2 Chapter 4 as published in Animals

animals

MDPI

*Article*

# Supplementation of Spring Pasture with Harvested Fodder Beet Bulb Alters Rumen Fermentation and Increases Risk of Subacute Ruminal Acidosis during Early Lactation

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Received: 25 May 2020; Accepted: 27 July 2020; Published: 30 July 2020

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**Simple Summary:** Fodder beet (FB) is widely used in grazing dairy systems of New Zealand to support early- and late-lactation milk production, however, the large fraction of water-soluble carbohydrate present in FB bulbs presents a risk of subacute and acute ruminal acidosis. Despite widespread use of FB across New Zealand, the incidence of ruminal acidosis using industry-recommended methods of feeding FB has not been investigated. This study analyzed the time-dependent changes to rumen fermentation, apparent dry matter intake, milk production, milk composition and plasma amino acid concentration of grazing dairy cows supplemented with moderate amounts (40% of dry matter intake) of FB during early lactation. Our findings indicate that incidence of subacute ruminal acidosis due to FB is greater than currently realized, as 25% of cows developed severe subacute ruminal acidosis following transition to target FB allocation (40% of daily intake). Across all cows, FB reduced rumen pH, feed conversion efficiency and was not advantageous to milk production. These results suggest methods for adapting cows to a diet containing FB require further evaluation to reduce the risk of subacute ruminal acidosis (SARA) experienced by individuals within the herd.

**Abstract:** In a cross-over design, eight rumen cannulated dairy cows were used to explore the industry-recommended method for dietary transition to fodder beet (FB: *Beta vulgaris* L.) on changes to rumen fermentation and pH, milk production, dry matter intake (DMI) and the risk of subacute ruminal acidosis (SARA) during early lactation. Cows were split into two groups and individually allocated a ryegrass (*Lolium Perenne* L.) and white clover (*Trifolium repens* L.) diet (HO) or the same herbage supplemented with 6 kg DM/cow of harvested fodder beet bulbs (FBH). Dietary adaptation occurred over 20 days consisting of: stage 1: gradual transition to target FB intake (days 1–12, +0.5 kg DM of FB/d); stage 2: acclimatization (days 13–17) and stage 3: post-adaption sampling (days 18–20). Response variables were analyzed as a factorial arrangement of diet and stage of adaption using a combination of ANOVA and generalized linear mixed modelling. Dietary proportion of FB represented 22, (stage 1), 32 (stage 2) and 38% (stage 3) of daily DMI. One cow during each period developed SARA from FB and the duration of low pH increased with FBH compared to the HO treatment ($p < 0.01$). Rumen concentrations of lactic and butyric acid increased with FBH but concentrations of acetate, propionate and total volatile fatty acids (VFA) declined by 9.3% at day 20, compared to the HO treatment ($p < 0.01$). Treatments did not affect milk production but total DMI with supplemented cows increased during the final stage of adaptation and feed conversion efficiency (FCE kg milk/kg DM) declined with the FBH treatment. The occurrence of SARA in 25% of animals fed FB suggest it is a high-risk supplement to animal health and further evaluation of industry-recommended methods for feeding FB at the individual- and herd-scale are needed.

# A.3 Chapter 6 as published in Livestock Production Science

Livestock Science 241 (2020) 104225

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

## In vitro fermentation of fodder beet root increases cumulative gas production of methane and carbon dioxide.

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ARTICLE INFO

*Keywords:*
Fodder beet, In vitro
Gas production, Ruminant nutrition
Enteric methane
Carbon dioxide

ABSTRACT

The relationship between proportion of fodder beet root (FB: *Beta vulgaris* subsp. *vulgaris* L.) in a perennial ryegrass (*Lolium perenne*) diet and gas production was investigated in vitro. Cumulative gas production and fermentation products were measured in calibrated glass syringes using a factorial arrangement (4 x 6), of dry matter proportion of FB (0, 15, 35 and 50% DM: 0FB, 15FB, 35FB and 50FB, respectively), and incubation time (0, 2, 4, 8, 12 and 24 h). Concentrations of methane ($CH_4$) and carbon dioxide ($CO_2$) produced both in fermentation and from bicarbonate buffering of volatile fatty acid, were also calculated. The proportion of FB root linearly increased volume of gas (mL) produced per g dry matter (DM) and per g organic matter (OM) with gas accumulation 14.8% greater for 50FB compared to 0FB at 24-hours of incubation ($P < 0.001$). The concentration of propionic and butyric acids also increased relative to the proportion of FB ($P < 0.001$). Stoichiometric calculations indicate that FB increased $CO_2$ released from both buffering and fermentation processes but reduced the fractions of methane in gas (17.6, 17.4, 17.0 and 16.8 ± 0.4% for 0FB, 15FB, 35FB, and 50FB, respectively, $P < 0.001$). However, the greater OM content of FB increased the total volume of both $CO_2$ and $CH_4$ produced. Results suggest that FB altered the thermodynamics of fermentation early in digestion by increasing formation of propionate which acted as a hydrogen sink to reduce formation of methane. However, as fermentation progressed, butyrate forming pathways became more favourable. The greater OM content of FB compared to ryegrass increased absolute volumes of $CO_2$ and $CH_4$ from fermentation, resulting in greater emission of gasses per kg DM of substrate. Further investigation in vivo is required to determine potential effects of microbial protein synthesis on gas production and further verify these results.

### 1. Introduction

The rapid incorporation of FB into pastoral grazing systems of New Zealand has preceded research of its impact on ruminant production. While altered rumen fermentation profiles and changes to milk production and composition have been reported (Fleming et al., 2018; Waghorn et al., 2018, 2019; Dalley et al., 2019), enteric gas production from a diet containing a mixture of perennial ryegrass (PRG) and FB has had minimal attention.

Methane and $CO_2$ are potent greenhouse gases (GHG) that are produced from microbial fermentation of carbohydrates and amino acids (Johnson and Johnson, 1995). The 2017 report of the Ministry for the Environment estimated that agriculture was responsible for 46% of New Zealand's GHG emissions. Given social and environmental pressure, development of feeds and feeding regimes which limit GHG emissions is crucial to the progression of agriculture. Additionally, the process of enteric $CH_4$ production is an inefficient conversion of energy, representing approximately 2–12% of GE which is un-utilised by the ruminant for maintenance, growth and lactation (Johnson and Johnson, 1995; Howden and Reyenga, 1999). Formation of $CH_4$ is dependant on the availability of hydrogen (H) in the rumen, which is a product of animal intake and the chemical composition of the diet (Johnson and Johnson, 1995). While microbial production of $CH_4$ is the most prolific H sink, formation of propionate is also a competitive pathway for H use. Alternatively, formation of acetate and butyrate result in the net release of H and subsequently increase enteric $CH_4$ emissions from livestock (Johnson and Johnson, 1995). Analysis of the volatile fatty acid (VFA) profiles of cows fed FB suggest the concentration of butyrate and propionate increase when FB is used to supplement a total mixed ration diet (Eriksson et al., 2004). While Jonker et al. (2016) reported daily $CH_4$ emissions (g $CH_4$/kg DMI) declined 28% in non-lactating cows fed ~50% FB and 50% pasture

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https://doi.org/10.1016/j.livsci.2020.104225
Received 21 February 2020; Received in revised form 5 August 2020; Accepted 22 August 2020
Available online 22 August 2020
1871-1413/ 

## A.4 Chapter 7 as published in The Journal of Agricultural Science

The Journal of Agricultural Science

cambridge.org/ags

Animal Research Paper

Cite this article: Fleming AE, Dalley D, Bryant RH, Edwards GR, Gregorini P (2020). Modelling feeding strategies to improve milk production, rumen function and discomfort of the early lactation dairy cow supplemented with fodder beet. The Journal of Agricultural Science 1–13. https://doi.org/10.1017/S0021859620000593

Received: 19 February 2020
Revised: 28 May 2020
Accepted: 13 July 2020

Key words:
Fodder beet; milk production; minimum total discomfort; modelling; sub-acute ruminal acidosis

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# Modelling feeding strategies to improve milk production, rumen function and discomfort of the early lactation dairy cow supplemented with fodder beet

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## Abstract

Feeding fodder beet (FB) to dairy cows in early lactation has recently been adopted by New Zealand dairy producers despite limited definition of feeding and grazing management practices that may prevent acute and sub-acute ruminal acidosis (SARA). This modelling study aimed to characterize changes of rumen pH, milk production and total discomfort from FB and define practical feeding strategies of a mixed herbage and FB diet. The deterministic, dynamic and mechanistic model MINDY was used to compare a factorial arrangement of FB allowance (FBA), herbage allowance (HA) and time of allocation. The FBA were 0, 2, 4 or 7 kg dry matter (DM)/cow/day (0FB, 2FB, 4FB and 7FB, respectively) and HA were 18, 24 or 48 kg DM/cow/day above ground. All combinations were offered either in the morning or afternoon or split across two equal meals. Milk production from 2FB diets was similar to 0FB but declined by 4 and 16% when FB increased to 4 and 7 kg DM, respectively. MINDY predicted that 7FB would result in SARA and that rumen conditions were sub-optimal even at moderate FBA (pH < 5.6 for 160 and 90 min/day, 7FB and 4FB respectively). Pareto front analysis identified the best compromise between high milk production and low total discomfort was achieved by splitting the 2FB diet into two equal meals fed each day with 48 kg DM herbage. However, due to low milk response and high risk of acidosis, it is concluded that FB is a poor supplement for lactating dairy cows.

## Introduction

In the last 5 years, fodder beet (Beta vulgaris; FB) has been widely adopted as a winter forage crop or as a supplement for early and late-lactation dairy cows in ryegrass pasture-based (Lolium perenne) systems of New Zealand. Although the use of FB has declined slightly within the last 2 years, popularity of FB has previously been driven by the high yield potential >20 t dry matter (DM)/ha (Chakwizira et al., 2013, 2016), high crop utilization >90% (Saldias and Gibbs, 2016) and the versatility to graze FB in autumn and winter or harvest bulb to supplement herbage in spring. FB is rich in water soluble carbohydrate (WSC) which accounts for over 60% of the biomass (Clark et al., 1987) and contains minimal proportions of fibre (<200 g/kg DM) and crude protein (CP; <100 g/kg DM) (Dalley et al., 2017). The low CP and high WSC content of FB bulb may reduce N excretion by diluting intake of high CP herbage (Dalley et al., 2019), or by synchronizing the supply of WSC to soluble protein. Nutrient synchrony may improve microbial utilization of soluble protein and reduce the excretion of urea in urine, which contributes to eutrophication and N leaching (Hall and Huntington, 2008; Cameron et al., 2013). However, the large fraction of sugar and low fractions of fibre also present a risk of sub-acute ruminal acidosis (SARA) and acute ruminal acidosis (Owens et al., 1998). Acidosis occurs when volatile fatty acids (VFAs) rapidly accumulate in the rumen, causing pH to decline and limiting microbial degradation of cellulose and fibre (Khafipour et al., 2009). SARA is characterized by daily episodes of low pH and reduced buffering capacity (Owens et al., 1998), but is self-corrected. Declining pH proliferates microbes that produce lactic acid which is 10-fold more acidic than other VFAs (Owens et al., 1998). Increased lactic acid causes a downward spiral of rumen pH leading to acute and often systemic acidosis that the animal is unable to self-correct.

Although acute presentation represents an immediate loss of capital to the dairy business, monetary losses from SARA are not obvious but may be equally financially harmful due to the limited diagnostic ability and reported wide-spread prevalence across the herd (Plaizier et al., 2008). Animals suffering from SARA experience welfare challenges such as reduced intake, malaise and translocation of endotoxin present in the cell wall of Gram-negative

# A.5 Chapter 8 as published in Agricultural Systems

Agricultural Systems 187 (2021) 102993

Contents lists available at ScienceDirect

Agricultural Systems

journal homepage: www.elsevier.com/locate/agsy

## Fodder beet to support early and late lactation milk production from pasture, is it worth the risk?

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ARTICLE INFO

*Keywords:*
Fodder beet
Grazing dairy cows
Farm profit
Grazing dairy systems
Ruminal acidosis
Complementary forage crops

ABSTRACT

High yielding crops such as maize (*Zea mays L.*) and fodder beet (FB; *Beta vulgaris L*), are commonly used to extend lactation and increase animal productivity from pastoral dairy systems. Financial modelling to compare costs and benefits of different crops is useful for decision making, but such modelling often fails to account for potential animal health risks which can be associated with feeding supplements. A multi-component, whole-farm modelling approach was used to predict milk solids (MS, milk fat + protein) production and the economic farm surplus (EFS: operating surplus – adjustments) between 2016 and 2018 for an irrigated farm in Canterbury (South Island) and a non-irrigated farm in the Waikato (North Island), of New Zealand. The financial risk of the dairy business was measured using the ratio between mean return on assets (ROA) minus an assumed 5% risk-free ROA, and the standard deviation of ROA was calculated from 300 combinations of climate, milk, and feed price, land appreciation, and interest rate. Four scenarios of autumn and spring supplementation of pasture were considered at each geographical location; imported maize silage (Base), a crop of maize silage grown on the milking platform (MSC; area used to produce milk), a crop of FB grown on the milking platform (FBC), and a FB crop with an outbreak of acute (1% stock fatality) and subacute ruminal acidosis (5% decline of feed intake) across the entire herd (FBAC). The MSC scenario improved EFS by 5.8% compared with Base in both the irrigated and the dryland system. The predicted response to MSC reflected greater milk production, lower feed expenses, and shorter crop rotation, compared with either Base, FBC, or FBAC. While FBC increased EFS by 4.8% compared with Base under irrigation, EFS was similar to Base under dryland conditions ($2711 and $2759/ha, respectively). The limited advantage of growing FB under dryland conditions reflect reduced herbage supply due to the extended crop duration of FB compared with maize silage. Model predictions suggest that FBAC will increase the financial risk by reducing milk production and EFS by 6.5% (irrigated) and 7.1% (dryland) compared with Base. In the absence of any adverse health risks, farm performance from the FBC scenario was comparable to that of MSC under irrigated conditions. However, in dryland conditions, and when the potential economic cost of acute and sub-acute ruminal acidosis is considered, there is little advantage to growing FB on the milking platform.

### 1. Introduction

Pastoral dairy production systems in NZ are heavily reliant on traditional perennial ryegrass (*Lolium perenne*; PRG) and white clover (*Trifolium repens*; WC) swards to meet the energy demands of dairy cattle. While PRG × WC swards are an inexpensive and simple combination of pasture species to graze, additional inputs (imported feed or forage crops) are needed to meet animal requirements between late autumn and spring, with additional feed inputs or irrigation required in areas that are prone to drought conditions during summer months (Clark et al., 2001; Neal et al., 2009). The heavy reliance on PRG × WC herbage for milk production and the deregulated nature of the NZ dairy industry expose NZ dairy producers to several business risks which include environmental, input, and output price, climate, biological, and production risks (Chapman et al., 2007). The combination of high relative costs for feed supplements, extreme market fluctuations and homogeneity of income (milk sales represent more than 90% of net income), constrain operating profit and resilience of New Zealand dairy businesses (McCall and Clark, 1999; Neal and Roche, 2020). System intensity of NZ dairy systems are defined by the amount of feed that is

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https://doi.org/10.1016/j.agsy.2020.102993
Received 20 May 2020; Received in revised form 5 November 2020; Accepted 10 November 2020

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